The slaughterhouse as data source for monitoring programmes in cattle

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1 Abbreviations

AMD: animal movement database

BT: Bluetongue

bTB: bovine tuberculosis

CAC: Codex alimentarius Comission

EBL: Enzootic bovine leucosis

ELISA: Enzyme Linked Immunosorbent Assay

ESBL: extended-spectrum β-lactamase

EU: European Union

FSVO: Food Safety and Veterinary Office

IBR: Infectious bovine rhinotracheitis

ICC: ingoing contact chain

ID: in-degree

IPPC: International Plant Protection Convention

OCC: outgoing contact chain

OD: out-degree

OIE: World organization of animal health

SNA: social network analysis

SPS Agreement: Agreement on the Application of Sanitary and Phytosanitary Measures

VPH: Veterinary Public Health

WHO: World Health Organisation

WTO: World Trade Organisation

2 Summary

The encountered animal and public health related challenges over the last decades, with a globalized market and current changes in climate, there is general consensus that the (re-) emergence of infectious disease is going to be a major preoccupation of veterinary public health also in the future. To protect domestic livestock from the introduction of diseases and to facilitate trade, countries are bound by international agreements to conduct nation-wide surveillance programmes with a sound scientific basis in order provide evidence on the sanitary status of the production animals.

The contrasting scarce resources at disposition give flexible and cost effective tools and methods for animal health surveillance great relevance.

In Switzerland, the implementation of bulk tank milk testing yields a significant potential for reducing costs and effort of surveillance programmes, as on-farm blood sampling is laborious and costly. On the downside, cattle reared for meat production, i.e. roughly ⅓ of the Swiss cattle population, are not covered. An alternative for this population segment would be the sampling in slaughterhouses, provided the abattoir cattle population reflects the general cattle population in the surveyed area.

Based on the data from the Swiss animal movement database (AMD) and a feasibility study in the six biggest slaughterhouses, the aim of the present PhD project was to assess the practicability, cost-effectiveness and representativeness of sampling the non-dairy population at the slaughterhouse instead of on-farm.

A part of the study was to understand the demography and movement patterns in the Swiss cattle industry. A stratified demographic population model served to define parameters that can describe the population dynamics in dairy and non-dairy cattle. By introducing trigonometric functions to simulate the seasonal oscillations of calve birth and mortality rates, the model fitted very well to the data from the AMD. The

obtained population parameters can be used to describe the population dynamic in cattle as building block for future model applications. The network analysis of the cattle movements depictured a highly interlinked industry with the properties of a scale free network and very large contact chains (i.e. chronological movement sequences). The seasonal changes in the network are linked to the traditional alpine pasturing during the summer months.

Possible risk factors for surveillance would be a high level of ingoing contacts (direct or over several farms) but as the cattle trade network is so connected and many traders operate on national level the value of the risk factors compared to random sampling should be validated in further studies. To assess also the connectivity of the network, the movements were not only investigated using network analysis techniques but also by mapping them on the road system in Switzerland. The intensity of road utilization creates a distinct pattern and allows identifying high risk areas for disease spread.

The sampling at the slaughterhouse was studies from different points of view; a transdiciplinary approach was realized to assess the practicality and implications for the veterinary service if sampling is shifted from on-farm to the slaughterhouse. By including the chief meat inspectors of the six biggest cattle slaughterhouses in Switzerland, the experience and hands-on knowledge of the people who are most concerned by the implementation of such a shift in sampling policy could be integrated from the beginning of the project.

Risk factors derived from the animal movement database for the occurrence of extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* in young cattle in Switzerland were analysed using a slaughterhouse sample. The faeces samples could be gained at the slaughterhouse without problems and represented the Swiss cattle

population well. Risk factors for shedding ESBL producing *Enterobacteriaceae* were age, primary production type, meaning dairy compared to beef on farm of origin the number of animal movements to the farm of origin. The gained intelligence could improve management strategies in animal holdings towards a reduction of ESBL prevalence in cattle.

With a more theoretical approach, the sampling was modelled using the Swiss cattle movement data from 2012 for different scenarios. With this individual-based model, the costs and sensitivity of the sampling programme was compared for on-farm versus slaughterhouse sampling. The model showed that on animal level the slaughterhouse was cheaper than on-farm sampling with the same outcome. For conclusions on herd-level, the low herd-level sensitivity at the slaughterhouse is limiting possible sampling scenarios. For instance, to proof absence from disease with the internationally given 99% sensitivity, that the herd-level prevalence is below 0.2%, the samples need to be collected over the entire year with a limited number of samples per day to cost less than the on farm sample with the same outcome.

In conclusion, sampling at slaughterhouse level is a valid alternative to on-farm blood sampling and the slaughterhouses provide a flexible sampling location where hundreds of specimens are accessible daily. For farm-level outcomes of routine surveillance, the programme must be planed carefully and a longer sampling period is necessary to reach reasonable system sensitivities. The practical feasibility is given, as long as all stakeholders are included in the planning and implementation of surveillance programmes.

The animal movement database is a valid resource for a wide range of information related to cattle production and trade for the public health sector. The conducted

network analysis and the dynamic population model gave insight to some characteristics of the Swiss cattle industry and create a basis for further exploration of disease transmission and control strategies in the cattle population.

3 Introduction

The public veterinary service operates on the interface of animal and human health. Although the focus lies on keeping the livestock in a good sanitary status, it is the human population that benefits from freedom of diseases transmissible from animals to humans, a prosperous agriculture, safe food products of animal origin and a flourishing trade of animals and animal products with other countries. The WHO defines Veterinary Public Health (VPH) in 1999 as "*the sum of all contributions to the physical, mental and social well-being of humans through an understanding and application of veterinary science*" (WHO n.d.).

Historically, economic losses through infectious diseases of the livestock like Rinderpest, Anthrax and swine erysipelas were the driving cause for the evolution of the modern veterinary services in Europe (Thrusfield 2005). With population growth and numbers of wars fought in the 17th and 18th century, diseases spread more easily and prevalences in livestock increased. The failure to control these devastating outbreaks pushed efforts towards understanding the cause and find treatments which eventually led to the discovery of bacteria and viruses as causal agents of infectious diseases in animals. With the development of diagnostic tools and the discovery of efficient antibiotic medicine and vaccines, the discipline of veterinary epidemiology and state veterinary services evolved. In the second half of the last century, the shift from treating individual animals to large-scale monitoring of populations took place (Thrusfield 2005; Schönherr 1991; Doho et al. 2009; Schwabe 1993). During the same period, the divergence of veterinary and human medicine became a rising concern (Schwabe 1984). Over the last decades, concepts of „OneHealth" and „EcoHealth" emerged, stressing that human and animal health are closely interlinked and further entwined with the ecosystem they live in (Zinsstag 2012). Within this

broad conceptual framework, the relationship of humans and its livestock has an important part. The close contact of humans and animals and the high density of animals in livestock production facilitate the transmission of diseases between animals and between animals and humans (e.g. Enserink 2010). A close collaboration of veterinary and human medicine in case of zoonotic outbreaks could diminish the time lag between the first detection of syndromes (in either animals or humans) and the diagnosis and therefore the initiation of effective control measures (Zinsstag et al. n.d.).

State veterinary services have an essential role in protecting the general public from zoonotic disease. Half of the human known pathogens and 75% of emerging diseases are transmissible from vertebrate to humans and vice versa (Sargeant 2008). Beside the risk of the introduction of zoonotic diseases (Taylor et al. 2001), outbreaks in production animals can have severe economic consequences (Pimentel et al. 2001; Thompson et al. 2002).

In times of worldwide international trade, climate change and increased human traffic, the risk of disease introduction in a previous free area is a constant threat. Not only poses the trade with live animal and animal products a risk of global disease spread (Stärk 2010; Nesbakken 2009), but change in climate is suspected to alter the distribution of arthropod vectors and their associated agents (Githeko et al. 2000; Purse et al. 2005).

The necessity to protect the domestic production animals from the introduction of diseases led to international standards and agreements. For instance, the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) entered into force in 1995. The main goal of the agreement is to facilitate and ensure fairness in trade, allowing the countries to protect the domestic human,

livestock and plant health status. It is based on the standards of the Codex alimentarius Comission (CAC), the World organization of animal health (OIE) and the International Plant Protection Convention (IPPC) (WTO n.d.).

The SPS agreement states in Article 2 *"Members shall ensure that any sanitary or phytosanitary measure is applied only to the extent necessary to protect human, animal or plant life or health, is based on scientific principles and is not maintained without sufficient scientific evidence, except as provided for in paragraph 7 of Article 5".*

To provide evidence on the sanitary status of domestic livestock, countries are bound to conduct nation-wide surveillance programmes with a sound scientific basis (Reist et al. 2012; Hadorn et al. 2002). The recent trends in surveillance of livestock and their application for the surveillance and monitoring of the cattle population in Switzerland is discussed in the section 4.2 but first, for a better understanding of the context, sector 4.1 describes the veterinary service and the Swiss cattle industry.

3.1 Stakeholders and instruments for cattle surveillance in Switzerland

3.1.1 International background and legal framework

Traditionally, animal diseases were monitored passively by mandatory notification system for listed diseases. Since the SPS agreement came into force in 1994, Switzerland incorporated active surveillance systems for the livestock population (Stark 1996).

With the formation of the European Union, the political landscape changed drastically for Switzerland. In 2002, the EU and Switzerland agreed on common veterinary area, regulated in Annex 11 of the Agreement on trade in agricultural products (Anonymous 2002). The objective is to facilitate trade by establishing a mechanism for the reciprocal recognition of the equivalence of legislation consistent with the protection of public and animal health. Annex 11 determines also the mandatory surveillance and monitoring programmes and either directly specifies their technical application or refers to the relevant EU regulatory guidelines and directives (Reist et al. 2012). Swiss legislation lists and classifies epizootic diseases if they are of national concern (Anonymous 1966), i.e. if they

i. are zoonotic
ii. are not controllable by individual animal keepers and without the intervention of several animal holdings
iii. are dangerous for indigenous wild animals
iv. are associated with important economic consequences
v. are relevant for international trade with animals or animal products

3.1.2 The Swiss veterinary service

Switzerland is a confederation that consists of 26 cantons (figure 1). The cantons are responsible for the implementation of federal and cantonal law. Every canton has its own constitution and its own government. This is reflected in the federal organisation of the Veterinary service; the central authority (Federal food safety and veterinary office, FSVO) collaborates with 23 cantonal veterinary services to ensure food safety, animal health and animal welfare in primary production (FFCU 2013).

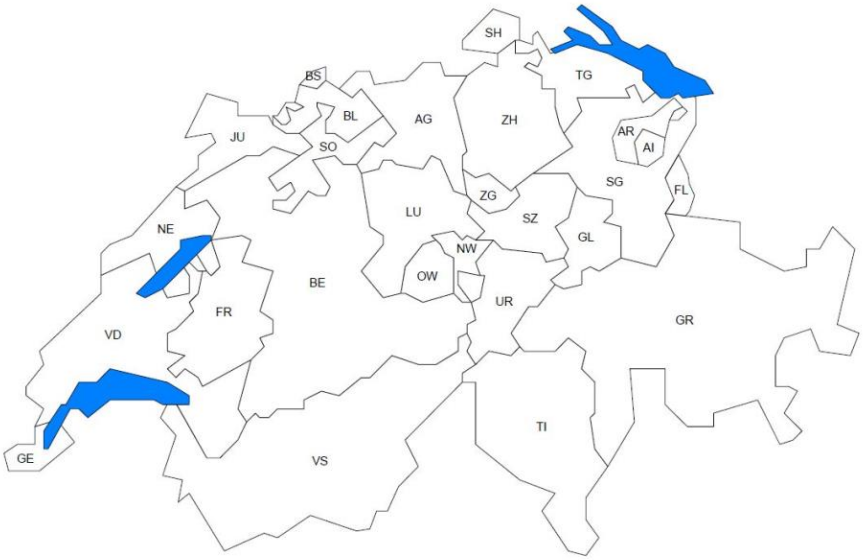

Figure 1: The Swiss cantons.

Aargau AG/ Appenzell- Ausserrhoden AR/ Appenzell- Innerrhoden AI/ Basel- Landschaft BL/ Basel- Stadt BS/ Bern BE/ Fribourg FR/Genève GE/ Glarus GL/ Graubünden GR/ Jura JU/ Luzern LU/ Neuchâtel NE/ Nidwalden NW/ Obwalden OW/ St. Gallen SG/ Schaffhausen SH/ Schwyz SZ/ Solothurn SO/ Thurgau TG/ Ticino TI/ Uri UR/ Valais VS/ Vaud VD/ Zug ZG/ Zürich ZH/ Fürstentum Liechtenstein FL (source: FSVO, Evaluation of the Swiss Veterinary service, 2009).

The monitoring of the health status of livestock is based on three pillars; prevention, early detection and active surveillance. Preventive measures include the maintaining of disease awareness of animal keepers, enforcing hygienic and animal friendly husbandry, supporting the private animal health services and by animal movement controls. Controls in primary production, i.e. farm visits, slaughterhouses and meat inspection are under the authority of the cantonal veterinary services.

The FSVO is responsible for the planning and implementation of national surveillance programmes. The on-farm sampling itself is organized by the cantonal veterinary services and conducted by official veterinarians (FVO 2009).

3.1.3 Cattle farming in Switzerland

Cattle are the major livestock species in Switzerland and contribute considerably to the national identity. 70 % of the agricultural area is grassland and used as feeding ground for cattle (FSO n.d.). About 1.6 Mio cattle are kept on 42’000 farms, 2/3 of which are dedicated to dairy production. Due to direct payments, i.e. financial compensation for services provided by farmers for the common good small scale farming is possible and predominating in Switzerland (mean herd size ~40 cows). In 2012, the Swiss government invested 2.8 billion CHF in direct payment. Animal friendly farming and grassland based milk and meat production is supported financially. Pasturing of dairy cattle and suckler cow husbandry are therefore common practices. To maintain the touristic merchandized Swiss landscape with open montane grassland, alpine pasturing is also subsidized (FOAG 2014). One fourth of the cattle population spends the summer month on alpine pastures (figure 2 a) and b)).

Cheese and chocolate export are important sources of revenue for the Swiss agriculture and 80% of exported dairy products go to EU countries (FOAG 2013).

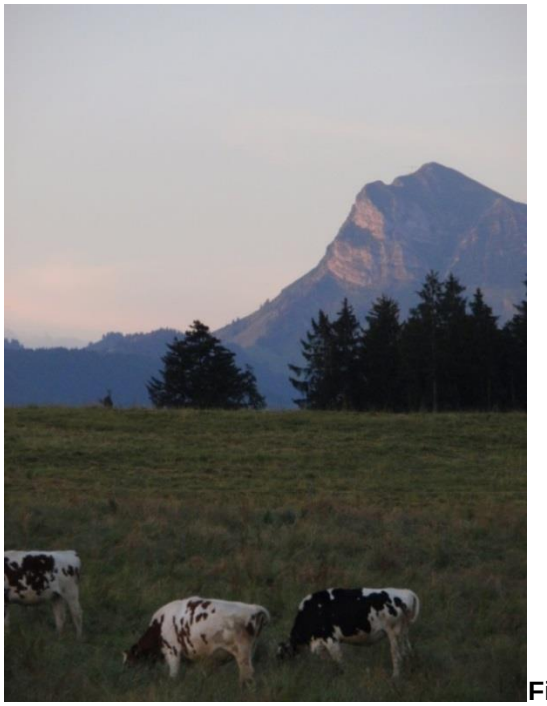

Figure 2a: alpine pasture in Gibloux, canton Fribourg

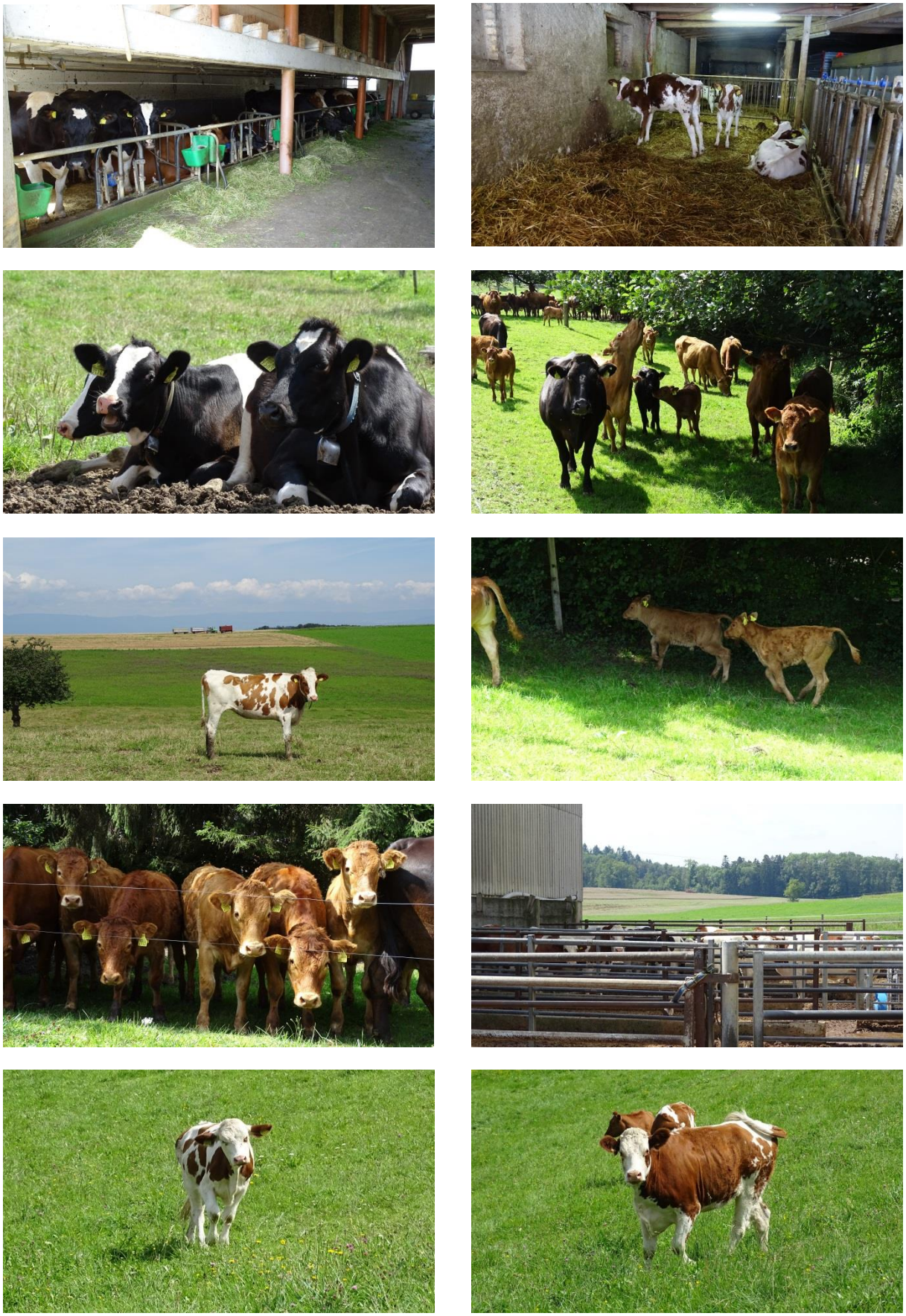

Figure 2b: Impressions of Swiss cattle in Corserey, canton Fribourg.

3.1.4 The Swiss cattle registry

It was mainly the BSE crisis in the late 20th century and the thereby lost trust of consumers in the meat industry that led to the mandatory implementation of nationwide cattle registry databases in the EU (McGrann & Wiseman 2001; Shanahan et al. 2009). The aim of such registries is the traceability of food and foodstuff and the prevention and the facilitation of the control of diseases (McKean 2001). In Switzerland, the animal health legislation was reformed in 1998 and in the context of the BSE crisis the legal basis for an animal movement registry was created (Lüdi 2004).

In 1999 an electronic registry of cattle and cattle holdings was introduced in conformity with the Agreement on trade in agricultural products (Anonymous 2002), stating Switzerland is bound to have a legislation covering sanitary measures applicable to trade in live animals and animal products that is equivalent to EU law. The Swiss animal movement database (AMD) was developed constantly since (Lüdi 2004; Anonymous n.d.).

It contains a central register of all holdings on which biungulates or equids are kept, slaughtered or traded. Cattle are tagged with official ear tags by birth and all movements must be registered with the database by the animal keeper. The animal keepers must keep an up-to-date list of all cattle with details of the animals kept at their business and must complete an accompanying document whenever biungulates are moved. The export, change of ownership, change of stable, slaughter or death must be reported to the AMD.

Besides plausibility checks of the reports by animal keepers by the AMD, compliance with the regulations is controlled as part of official veterinary inspections (FFCU 2013).

3.2 Principle of livestock surveillance and their implementation for cattle in Switzerland

3.2.1 Concepts and terminology used in the animal health field for surveillance

The International Conference on Animal Health Surveillance (ICAHS) in Lyon 2011 made the effort to provide definitions for terms related to animal health surveillance (Hoinville et al. 2013).

Table 1 provides an extract of said definitions that are relevant for the present document.

Table 1: Definitions of selected terms related to animal health surveillance from the ICAHS report ‚Animal Health Surveillance Terminology – Final Report from Pre-ICAHS Workshop, July 2013‘

Term	Definition
Surveillance	The systematic, continuous or repeated, measurement, collection, collation, analysis, interpretation and timely dissemination of animal health and welfare related data from defined populations. These data are then used to describe health hazard occurrence and to contribute to the planning, implementation, and evaluation of risk mitigation actions
Monitoring[1]	The systematic, continuous or repeated, measurement, collection, collation, analysis and interpretation of animal health and welfare related data in defined populations when these activities are not associated with a pre-defined risk mitigation plan although extreme changes are likely to lead to action.
Surveillance purpose	Describes the type of information that will be obtained about the occurrence of a health hazard using a particular surveillance activity **Early detection / warning** of known (exotic or re- emerging) or unknown (new) disease **Substantiate freedom** from disease or infection **Describe the baseline level, distribution and impact** of specified disease(s)

[1] In the present document, Surveillance and Monitoring are not strictly used according to the given definiton but merely as synonyms

Term	Definition
	Describe changesin the health of the population, including changes in health indicators or in the occurrence of specified diseases **Describe changes that might threaten the health** of the population, this may include changes in the population structure or in its exposure to risk factors **Detect cases** of diseases that are currently present to allow action to be taken to control disease
Risk-based surveillance	Use of information about the probability of occurrence and the magnitude of the biological and/or economic consequence of health hazards to plan, design and/or interpret the results obtained from surveillance systems.
Risk-based sampling	Designing a sampling strategy to reduce the cost or enhance the accuracy of surveillance by preferentially sampling strata (e.g. age groups or geographical areas) within the target population that are more likely to be exposed, affected, detected, become affected, transmit infection or cause other consequences (e.g. large economic losses or trade restrictions).
Active surveillance	Investigator-initiated collection of animal health related data using a defined protocol to perform actions that are scheduled in advance. Decisions about whether information is collected, and what information should be collected from which animals is made by the investigator
Passive surveillance	Observer-initiated provision of animal health related data (e.g. voluntary notification of suspect disease) or the use of existing data for surveillance. Decisions about whether information is provided, and what information is provided from which animals is made by the data provider.
Surveillance Component	A single surveillance activity (defined by the source of data and the methods used for its collection) used to investigate the occurrence of one or more hazards in a specified population
Unit of interest	Units selected for sampling in surveillance activity (level of sampling) E.g. animal, farm, batch, villag

3.2.2 Sensitivity from animal to herd-level and to overall surveillance system sensitivity

It is peculiar to veterinary epidemiology that the unit of interest is often not the individual animal but the herd. The herd in turn, must be defined according to natural circumstances[2]. Legal requirements for substantiating freedom from disease are often set on herd level.

But even if the herd is the unit of interest, analytic tests are often applied to the individual animal (i.e. blood sample) and inferences on the disease occurrence or absence are drawn on population level. The stepwise integration of information drawn on animal $\rightarrow$ herd $\rightarrow$ population level to reach desired confidence to infer on the disease status in a geographical region was subject of numerous studies. Martin et al. (1992) extended the concept of test sensitivity and specificity, applied to individuals, to the herd level providing formulas to calculate herd level sensitivity and specificity. Cameron & Baldock (1998) contributed formulas to compute the exact probability of detecting diseased animals for finite population. The use of pooled test to classify the status of a herd (diseased or not) is discussed by Christensen & Gardner (2000).

With the growing international interest in proofing the absence of a disease, several publications address the issue methodologically; Dufour et al. (2001) proposed general criteria to decide whether a territory is free of a disease. Cannon Cannon (2002; 2001) presents methods to demonstrate freedom from disease combining different surveillance components. Regarding the stepwise calculation of the confidence of a surveillance system he stated: *'the terms 'confidence' and 'sensitivity'*

[2] For this project, the herd is defined as the animals staying on one farm

are effectively synonymous. […] one level's confidence becomes the sensitivity used to calculate the next level's confidence.'

Martin et al. (2007) introduced scenario tree modelling for the integration of different data sources for the demonstration of freedom from disease.

3.2.3 Listed disease for cattle and their monitoring and surveillance

Table 2 gives an overview of the listed bovine diseases in Switzerland and the implemented federal surveillance. The country is free of most of the regulated diseases and therefore invests the biggest part of the available resources in substantiating freedom. Several recent research projects adopted the above described methods to optimize the sample size and therefore reduce cost of surveillance for proof of absence from disease.

Table 2: Listed diseases for cattle in Switzerland (Anonymous 1995) and the implemented monitoring or surveillance activities.

Disease	status[3]	MOSS
Bovine herpesvirus 1 (BoHV-1, IBR) Enzootic bovine leucosis (EBL)	Official free	Annual surveillance programme on farm level to prove absence of disease with bulk tank milk samples and on-farm blood samples
Tuberculosis	Official free	Meat inspection;
Bluetongue	Free	Annual surveillance programme on animal level to prove absence of disease with blood samples at the slaughterhouse
Bovine spongiform encephalopathy(BSE)		Fallen stock of adult cattle is examined systematically for BSE. Additionally, samples at slaughter from 7000 random selected animals that are older than 30 months are analysed
Bovine virus diarrhoea (BVD)		Eradication programme started 2008. In 2012, 99% of cattle farms were free of BVD. Since 2012, a surveillance programme with bulk tank milk samples and on-farm blood samples is in place.
Contagious bovine pleuropneumonia Lumpy skin disease Foot-and-mouth disease Rift valley fever Rinderpest Vesicular stomatitis Brucellosis Rabies	Official free	Passive surveillance: animal keepers/veterinarians notify suspicious cases
Campylobacter foetus	Free	
Anthrax		

[3] List of diseases Switzerland is free of (FSVO n.d.)

Disease	status[3]	MOSS
Besnoitiosis		
Tritichomonas foetus		
Botfly		
Leptospirosis		
Salmonellosis		
Blackleg		Passive surveillance: animal keepers/veterinarians notify suspicious cases
Campylobacteriosis		
Cryptospirodiosis		
Listeriosis		
Coxiellosis /Q-fever		
Neospora		
Paratuberculosis		
Toxoplasmosis		

3.2.3.1 Passive surveillance

Diseases that occur sporadically in Switzerland or are very unlikely to be introduced are monitored passively (see table 2). The FSVO strives to keep animal keepers informed and vigilant as disease awareness is the key to a sensitive passive surveillance (Hadorn et al. 2008).

Bovine tuberculosis (bTB) is considered an emerging zoonotic disease. Switzerland is officially tuberculosis free since 1960. After one random survey in 1997 to document freedom, the surveillance was reduced to passive abattoir and clinical surveillance (Hadorn & Stärk 2008; Schiller et al. 2011). In 2013, a cow with bTB was detected at the abattoir. Since then, about 7000 contact animals were tested and 7 farms were positive for bTB. In concordance with the findings of (Hadorn & Stärk 2008), disease awareness was actively promoted with meat inspectors (FSVO 2014a) and hunters (FSVO 2014b) to increase the sensitivity of the surveillance system.

3.2.3.2 Active surveillance

With the SPS agreement coming into effect, substantiating freedom from disease became the basis of international trade. EU law (Anonymous 2002) requires that it can be declared with 99 % reliability that less than 0,2 % of herds are infected with EBL or IBR. To reach this goal, using less resource possible, cost effective methods for the annual surveillance to substantiate freedom were explored in recent years. Applying the methods described by Cannon (2002), sample size reductions for the yearly repeated surveys could be obtained. By assessing the risk of introduction of EBL using a scenario pathway, the necessary confidence required to proof absence of disease could be lower for the current year taking into account the remaining confidence obtained by the survey of the previous year (Hadorn et al. 2002;

Schwermer et al. 2009). A similar approach was used to model required sample sizes for IBR (Knopf et al. 2007). Blickenstorfer et al. (2011) refined these methods, by including risk-based selection of herds in the calculation of the overall sensitivity and therefore reducing the sample size. 2011, bulk tank milk testing was introduced for the serological surveillance of dairy herds for EBL and adopted also for IBR in 2012 (FVO 2013a).

Bluetongue is an impressive example for how the epidemiologic situation can change quickly and that surveillance systems must be capable of adapting to changing circumstances (Reist et al. 2012). Since 2006, the Bluetongue surveillance changed from sentinel surveillance of the southern part of Switzerland to detect first incursion (Racloz et al. 2006) to surveillance for early detection of the entire country after the emergence of BTV-8 in central Europe with a combination of enhanced passive surveillance of small ruminants, vector surveillance and bulk milk testing of targeted cattle herds (Schwermer et al. 2008; Hadorn et al. 2009) and currently to animal level sampling in the slaughterhouses in accordance with EU legislation (Anonymous 2007), as after two mandatory vaccination campaigns bulk milk testing for antibodies is not informative regarding infection with BTV in adult cows anymore (Willgert et al. 2011).

BVD and BSE are both disease that are close to eradication in Switzerland and are now in the stage of surveillance for substantiating freedom (Häsler et al. 2011). BSE had an enormous impact on veterinary public health worldwide, and its erradication required substancial changes in the food safety policy (e.g. Vos 2000; Lloyd et al. 2006). While the ban of animal feed and the elimination of risk material from the food chain where general applied measures to stop the occurance of new cases, Switzerland was the first country to introduce an active monitoring programme

(Matthwes 2003). Today, fallen stock of adult cattle is examined systematically for BSE. Additionally, samples at slaughter from 7000 random selected animals that are older than 30 months are analysed.

The BVD eradication was economically motivated. Beginning in 2008, all cattle herds were systematically tested and positive animals were slaughtered (Presi & Heim 2010). In 2013, the herd-level prevalence of BVD was below 0.5% and the comprehensive testing of new born calves was replaced by a national, risk-based survey composed of bulk tank milk testing for dairy cattle and blood sampling for non-dairy herds (Reber et al. 2012).

3.3 Rationale and research framework

The encountered animal health related challenges over the last decades and the general consensus, that with a globalized market and change in climate the (re-) emergence of infectious disease is going to be a major preoccupation of veterinary public health also in the future (Steele 2008; Sargeant 2008), coupled with scarce resources at disposition, flexible and cost effective tools and methods for animal health surveillance are of great relevance.

Efforts to optimize surveillance programmes focused on reducing sample size by stratifying the population according the risk of disease occurrence (Stärk et al. 2006) and on the combination of different sources of information.

In Switzerland, the implementation of bulk tank milk testing yields a significant potential for reducing costs and effort (Schwermer et al. 2008; Reber et al. 2012) as on-farm blood sampling is laborious and costly.

On the downside, cattle reared for meat production, i.e. roughly ⅓ of the Swiss cattle population, young stock and bulls are not covered. When looking for PI-animals for the BVD surveillance or new infections of bluetongue in non-vaccinated animals, milk sampling is not an option.

However, for beef cattle the currently applied individual blood testing on farms brings some serious disadvantages. The husbandry of cattle for meat production involves far less human interaction than with dairy cows. It is also common to keep them in semi-wild conditions sometimes in considerable distances to the farms. This makes the sampling on the farm more time consuming and the sampling procedure itself difficult and at times dangerous as the animals are not used to close human contact and mother cows tend to defend their calves vigorously.

Also, as only one third of the population would have to be covered by on farm sampling, the cost efficiency is very low because the logistic and time effort is disproportionately high compared to the smaller number of farms to be sampled. In the view of the assumed further shortening of resources it is apparent, that a more cost-efficient method must be found.

An evident alternative would be the sampling in slaughterhouses, as it is done for surveillance programmes of pig and poultry (FVO 2013a; FVO 2013b) provided the abattoir cattle population reflects the general cattle population in the surveyed area. There are inherent reservations to which extent the abattoir population (offtake population) reflects the general population, as in general young male and older female (cows) are slaughtered (Ngandolo et al. 2009).This may introduce a bias if diseases are sex or age dependent (e.g. mastitis in young cows would be less frequently detected). If sufficiently large numbers of animals are sampled however, or if the age and sex of animals to be sampled can be pre-determined, abattoir sampling can be a valuable and cost-effective alternative for the surveillance of disease.

3.3.1 Building up basic knowledge

Despite the detailed data available through the animal movement database (AMD), in which births, deaths, slaughters and displacements of cattle and establishments that keep, handle, trade or slaughter cattle has been recorded for the last decades, analyses of the demographic structure, the distribution of the slaughtered cattle to the slaughterhouses or the cattle movements are lacking.

Therefore, effort was put in establishing a basis for future risk assessments, early detection and prophylaxis of animal disease and contingency plans, analyzing the available data thoroughly.

As part of this analysis, potential risk factors for disease transmission that can be red out of the AMD data were evaluated. The circumstances cattle live in can influence the probability of getting sick. Herd sizes, number of movements, production type (and inherent management practices) have all been linked as risk factors to disease (ref.) and were therefore studied in detail.

The importance of live animal movements for contagious disease spread is generally accepted (e.g. Wentholt et al. 2012). Information about animal movements and the contact network are therefore of great value to the public veterinary service.

Using epidemiologically relevant network indices, the type, spatial dimension and frequency of contacts (i.e. cattle movement) between establishments involved in today's cattle industry (farm, cattle dealer, markets, alpine pasturing during summer, slaughterhouse) can be identified and mathematically described. The farms and other establishments involved in cattle industry are represented as nodes, while the movement of cattle links the nodes (unidirectional, e.g. to the slaughterhouse or bidirectional, e.g. markets). The number of direct contacts of farms can be used to identify premises with an important role in the contact network and hence a priority for targeted surveillance (Nöremark et al. 2011).

3.3.2 Feasibility of slaughterhouse sampling in Switzerland

For animal diseases, the most commonly used diagnostic tools for antibody detecting are validated for blood serum. Against intuition, in slaughterhouses blood samples are hard to get. The moment of exsanguination is very critical, as it follows immediately the stunning. For not risking the animal to regain consciousness, no time loss can be afforded. Additionally, convulsions and uncoordinated pedaling of the animal as well as slippery floors pose a risk to the operator which should not be increased by supplementary assignments or disturbance by another person. Other

positions in the slaughter chain are possible to gain blood samples (blood that can be gained after opening the heart ventricles during meat inspection, big vessels on the carcass) and other sample materials (meat juice) are possible solutions.

Also, by transferring routine sampling from on-farm blood sampling to the slaughterhouse, a substantial work load is charged on the meat inspectors. To find practical solutions and estimate the added expenses in terms of labour time, the meat inspectors of the six biggest slaughterhouses were involved in a feasibility study.

3.3.3 Cost-effectiveness and possible sampling schemes of slaughterhouse sampling in Switzerland

In Switzerland, the slaughter pattern of cattle is complex compared to other farm animals; Cattle are not necessarily slaughtered in batches and commonly transport enterprises collect animals over the entire country to bring to the slaughterhouses. As a result, it is not predictable when, in which slaughterhouse and how many animals from a farm will be slaughtered. Compared to on-farm blood sampling, this makes the planning of surveillance activity very challenging. The confidence to detect diseased herds in the population depends on the herd-level sensitivity of each herd in the sample, which in turn is dependent on the number of animals sampled per herd (Martin et al. 1992; Cameron & Baldock 1998; Christensen & Gardner 2000). Contrary to on-farm sampling schemes, where sample size is determined before the start of the surveillance programme based on the desired confidence level (Schwermer et al. 2009), in a slaughterhouse sample the number of sampled animals per herd and the possible number of herds to sample depends on the decisions of the farmers. Different sampling schemes at the slaughterhouse were evaluated optimize the reached sensitivity by lowest possible sampling costs.

3.4 Literature

Anonymous, 2002. *Agreement between the European Community and the Swiss Confederation on trade in agricultural products.*,

Anonymous, 2007. Commission regulation 1266/2007 on implementing rules for Council Directive 2000/75/EC as regards the control, monitoring, surveillance and restriction on movements of certain animals of susceptible species in relation to bluetongue. *Official Journal of the European Communities*, L 283, pp.37–52.

Anonymous, 1966. *Tierseuchengesetz, SR 916.40*, Bern.

Anonymous, 1995. *Tierseuchenverordnung, SR 916.401*, Bern. Available at: http://www.admin.ch/opc/de/classified-compilation/19950206/index.html.

Anonymous, Verordnung vom 26. Oktober 2011 über die Tierverkehrsdatenbank (TVD-Verordnung) - Chronologie. Available at: http://www.admin.ch/opc/de/classified-compilation/20111006/history.html.

Blickenstorfer, S. et al., 2011. Using scenario tree modelling for targeted herd sampling to substantiate freedom from disease. *BMC veterinary research*, 7(1), p.49. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3170209&tool=pmcentrez&rendertype=abstract [Accessed May 27, 2013].

Cameron, A. & Baldock, F., 1998. A new probability formula for surveys to substantiate freedom from disease. *Preventive Veterinary Medicine*, 34(1), pp.1–17. Available at: http://apps.isiknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=3&SID=T1E4EnEpeDcn3FdfJ6k&page=1&doc=1 [Accessed April 25, 2013].

Cannon, R., 2001. Sense and sensitivity--designing surveys based on an imperfect test. *Preventive veterinary medicine*, 49(3-4), pp.141–63. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11311950.

Cannon, R.M., 2002. Demonstrating disease freedom — combining confidence levels. *Preventive Veterinary Medicine*, 52, pp.227–249.

Christensen, J. & Gardner, I.A., 2000. Herd-level interpretation of test results for epidemiologic studies of animal diseases. *Preventive veterinary medicine*, 45, pp.83–106.

Doho, I., Wayne, M. & Stryhn, H., 2009. *Veterinary Epidemiologic Research* 2nd ed., Charlottetown : VER Inc.

Dufour, B., Pouillot, R. & Toma, B., 2001. Proposed criteria to determine whether a territory is free of a given animal disease. *Veterinary Research*, 32, pp.545–563.

Enserink, M., 2010. Questions Abound in Q-Fever Explosion in the Netherlands. *Science*, 327, pp.266–267.

FFCU, 2013. *Multi-annual national control plan (MANCP)*, Bern. Available at: http://www.blv.admin.ch/blk/02557/index.html?lang=en&download=NHzLpZeg7t,lnp6I0NTU042l2Z6ln1ad1IZn4Z2qZpnO2Yuq2Z6gpJCEe3t_f2ym162epYbg2c_JjKbNoKSn6A--.

FOAG, 2013. *Agrarbericht 2013*, Bern.

FOAG, 2014. *Direktzahlungen in der Landwirtschaft.*, Bern.

FSO, Statistisches Lexikon der Schweiz. Available at: http://www.bfs.admin.ch/bfs/portal/de/index/infothek/lexikon/lex/0.topic.1.html.

FSVO, 2014a. *Handbuch Rindertuberkulose; Formen der Rindertuberkulose in der Fleischkontrolle.*, Bern.

FSVO, 2014b. *Handbuch: Tuberkulose beim Wild.*, Bern.

FSVO, *List of diseases Switzerland is free of*, Available at: https://www.google.com/url?q=http://www.blv.admin.ch/gesundheit_tiere/index.html?download=NHzLpZeg7t,lnp6I0NTU042l2Z6ln1ad1IZn4Z2qZpnO2Yuq2Z6gpJCEeoJ2e2ym162epYbg2c_JjKbNoKSn6A--&lang=en&sa=U&ei=Ms4-

VOnzOZaNuAS5nIHYBw&ved=0CAUQFjAA&client=internal-uds-cse&usg=AFQjCNFGP4A5E9O1zxm1p_qAC9HqqydUpg.

FVO, 2009. *Evaluation of the Swiss Veterinary Service*,

FVO, 2013a. *Jahresbericht Seuchenfreiheit 2012*, Bern.

FVO, 2013b. *Schlussbericht über das Überwachungsprogramm zum Vorkommen von niedrig pathogenen aviären Influenzaviren (LPAI) und Newcastle Disease Virus (NDV) bei Schweizer Nutzgeflügel 2012*, Bern.

Githeko, A. et al., 2000. Climate change and vector-borne diseases : a regional analysis. *Bulletin of the World Health Organization*, 78(9).

Hadorn, D. et al., 2009. Establishing a cost-effective national surveillance system for Bluetongue using scenario tree modelling. *Veterinary Research*, 40:57.

Hadorn, D. et al., 2002. Risk-based design of repeated surveys for the documentation of freedom from non-highly contagious diseases. *Preventive Veterinary Medicine*, 56, pp.179–192.

Hadorn, D., Haracic, S. & Stärk, K., 2008. Comparative assessment of passive surveillance in disease-free and endemic situation : Example of Brucella melitensis surveillance in Switzerland and in Bosnia and Herzegovina. *BMC Veterinary Research*, 4:52.

Hadorn, D. & Stärk, K., 2008. Evaluation and optimization of surveillance systems for rare and emerging infectious diseases. *Vet. Res.*, 39:57.

Häsler, B., Howe, K. & Stärk, K., 2011. Conceptualising the technical relationship of animal disease surveillance to intervention and mitigation as a basis for economic analysis. *BMC health services research*, 11(1), p.225. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3189394&tool=pmcentrez&rendertype=abstract [Accessed October 15, 2014].

Hoinville, L.J. et al., 2013. Proposed terms and concepts for describing and evaluating animal-health surveillance systems. *Preventive veterinary medicine*,

112(1-2), pp.1–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23906392 [Accessed October 19, 2014].

Knopf, L., Schwermer, H. & Stärk, K., 2007. A stochasticsimulation model to determine the sample size of repeated national surveys to document freedom from bovine herpesvirus I (BoHV-I) infection. *BMC Veterinary Research*, 3:10.

Lloyd, T.A. et al., 2006. Food scares, market power and price transmission: The UK BSE crisis. *European Review of Agricultural Economics*, 33, pp.119–147.

Lüdi, F., 2004. *Demographie, räumliche Verteilung und Dynamik der schweizerischen Rindviehpopulation in den Jahren 2002 und 2003*. Bern.

Martin, P.A.J., Cameron, A.R. & Greiner, M., 2007. Demonstrating freedom from disease using multiple complex data sources 1 : A new methodology based on scenario trees. , 79, pp.71–97.

Martin, S., Shoukri, M. & Thorburn, M., 1992. Evaluating the health status of herds based on tests applied to individuals. *Preventive Veterinary Medicine*, 14(1-2), pp.33–43. Available at: http://linkinghub.elsevier.com/retrieve/pii/016758779290082Q.

Matthwes, D., 2003. BSE: a global update. *Journal of applied Microbiology*, 94, p.120S–125S.

McGrann, J. & Wiseman, H., 2001. Animal traceability across national frontiers in the European Union. *Revue scientifique et technique (International Office of Epizootics)*, 20, pp.406–412.

McKean, J.D., 2001. The importance of traceability for public health and consumer protection. *REVUE SCIENTIFIQUE ET TECHNIQUE DE L OFFICE INTERNATIONAL DES EPIZOOTIES*, 20, pp.363–371.

Nesbakken, T., 2009. Food safety in a global market-Do we need to worry? *Small Ruminant Research*, 86, pp.63–66.

Ngandolo, R. et al., 2009. Comparative assessment of fluorescence polarization and tuberculin skin testing for the diagnosis of bovine tuberculosis in Chadian cattle. , 89, pp.81–89.

Nöremark, M. et al., 2011. Network analysis of cattle and pig movements in Sweden : Measures relevant for disease control and risk based surveillance. *Preventive Veterinary Medicine*, 99, pp.78–90.

Pimentel, D. et al., 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems and Environment*, 84, pp.1–20.

Presi, P. & Heim, D., 2010. BVD eradication in Switzerland--a new approach. *Veterinary microbiology*, 142(1-2), pp.137–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19883982 [Accessed June 5, 2013].

Purse, B. V et al., 2005. Climate change and the recent emergence of bluetongue in Europe. *Nature reviews. Microbiology*, 3, pp.171–181.

Racloz, V. et al., 2006. Establishment of an early warning system against Bluetongue virus in Switzerland. *Schweizer Archiv fur Tierheilkunde*, 148, pp.593–598.

Reber, A., Reist, M. & Schwermer, H., 2012. Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhino-tracheitis and bovine enzootic leucosis in Switzerland. *SAT*, 154(5), pp.189–197.

Reist, M., Jemmi, T. & Stärk, K., 2012. Policy-driven development of cost-effective, risk-based surveillance strategies. *Preventive veterinary medicine*, 105(3), pp.176–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22265642 [Accessed September 22, 2014].

Sargeant, J., 2008. The influence of veterinary epidemiology on public health : Past , present and future. *Preventive Veterinary Medicine*, 86, pp.250–259.

Schiller, I. et al., 2011. Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: Trade, surveillance and diagnostics. *Veterinary Microbiology*, 151, pp.153–159.

Schönherr, W., 1991. History of veterinary public health in Europe in the 19th Century. *Scientific and Technical Review of the Office International des Epizooties*, 10, pp.985–994.

Schwabe, C., 1984. *Veterinary medicine and human health*, Baltimore (USA): Williams & Wilkins.

Schwabe, C.W., 1993. The current epidemiological revolution in veterinary medicine. Part II. *Preventive Veterinary Medicine*, 18, pp.3–16.

Schwermer, H. et al., 2008. Das Überwachungsprogramm der Blauzungenkrankheit in der Schweiz. *Schweizer Archiv für Tierheilkunde*, 3, pp.129–132.

Schwermer, H., Reding, I. & Hadorn, D., 2009. Risk-based sample size calculation for consecutive surveys to document freedom from animal diseases. *Preventive Veterinary Medicine*, 92, pp.366–372.

Shanahan, C. et al., 2009. A framework for beef traceability from farm to slaughter using global standards : An Irish perspective. , 66, pp.62–69.

Stärk, K. et al., 2006. Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health : Review of current approaches. *BMC Health Services Research*, 6:20.

Stärk, K., 2010. Risks of animal-derived food in a global market--are we ready for the challenges? *Veterinary Journal*, 184(2), pp.121–123. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19695910 [Accessed October 15, 2014].

Stark, K.D.C., 1996. Animal health monitoring and surveillance in switzerland. *Australian Veterinary Journal*, 73, pp.96–97.

Steele, J., 2008. Veterinary public health: past success, new opportunities. *Preventive veterinary medicine*, 86(3-4), pp.224–43. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18417229 [Accessed October 14, 2014].

Taylor, L.H., Latham, S.M. & Woolhouse, M.E., 2001. Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 356, pp.983–989.

Thompson, D. et al., 2002. Economic costs of the foot and mouth disease outbreak in the United Kingdom in 2001. *Revue scientifique et technique (International Office of Epizootics)*, 21, pp.675–687.

Thrusfield, M., 2005. *Veterinary Epidemiology* 3rd ed., Oxford : Blackwell Science Ltd.

Vos, E., 2000. {EU} Food Safety Regulation in the Aftermath of the {BSE} Crisis. *Journal of Consumer Policy*, 23, pp.227–255. Available at: http://dx.doi.org/10.1023/A:1007123502914.

Wentholt, M. et al., 2012. Defining European preparedness and research needs regarding emerging infectious animal disease: results from a Delphi expert consulation. *Preventive Veterinary Medicine*, 103, pp.81–92.

WHO, World Health Organization. Available at: http://www.who.int/zoonoses/vph/en.

Willgert, K., Schroedle, B. & Schwermer, H., 2011. Spatial analysis of Bluetongue cases and vaccination of Swiss cattle in 2008 and 2009. *Geospatial health*, 5(2), pp.227–237.

WTO, The WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). Available at: http://www.wto.org/english/tratop_e/sps_e/spsagr_e.htm.

Zinsstag, J., 2012. Convergence of EcoHealth and One Health. *EcoHealth*, 9(4), pp.371–3. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3627853&tool=pmcentrez&rendertype=abstract [Accessed October 9, 2014].

Zinsstag, J., Waltner-Toews, D. & Tanner, M., Theoretical issues of One Health. In J. Zinsstag et al., eds. *One Health The Theory and Practice of Integrated Helath Approaches*. Marston Book Services Ltd.

4 Aims and objectives

4.1 Aim

The aim of the overall project is to investigate methods and developing concepts for an efficient and effective use of slaughterhouses as surveillance component for monitoring and control programmes on bovine diseases.

4.1.1 Objective 1: describe the Swiss cattle population using the data of the AMD.

- To describe the demographic composition and dynamics for the Swiss cattle population according to age cohorts and production system, including mortality, offtake to slaughter and birth rates
- To identify the contact network between establishments involved in the cattle industry.

4.1.2 Objective 2: To identify AMD derived risk factors for bovine MOSS and control programmes.

- Define risk-factors derived from network analyses of the AMD
- Define risk-factors for ESBL using the AMD in a prevalence study at the slaughterhouse

4.1.3 Objective 3: assess the feasibility, overall sensitivity and costs-effectiveness of using the slaughterhouse as data source in surveillance and monitoring programmes for cattle compared to “on farm” sampling.

- To assess possible matrices according to available diagnostic tests, cost and sampling effort, including the sampling point in the slaughterhouse.

- To assess the comparative surveillance sensitivity, representativeness and cost-effectiveness of slaughterhouse vs. “on farm” sampling.

5 Outline

The principle research question was the feasibility and cost efficiency of the shift from sampling non-dairy cattle on-farms, taking blood from the live animals to the slaughterhouse where the sampling could be assigned to the meat inspectors. The task was divided in three research questions;

- Is the sampling at the slaughterhouse feasible, are structural or technical changes necessary and what are the implication regarding resources, administration and organization for the meat inspectors
- Is the sample at the slaughterhouse representative of the cattle population and can the sample at the slaughterhouse meet the international standards for surveillance programmes regarding sensitivity
- Is the slaughterhouse a possible information source for risk factor analysis and consequently for risk based sampling

The feasibility was assessed and documented in collaboration with the six biggest Swiss slaughterhouses and the results were published in Fleischwirtschaft International 06/2013 (Chapter 8).

To assess the representativeness, the cattle population was analysed based on the animel movement data and described in two publications; , Demographic model of the Swiss cattle population for the years 2009-2011 stratified by gender, age and production type‘, PlosONE, 2014 (Chapter 6) and ‚Network analysis to inform risk based surveillance of cattle in Switzerland’ (submitted, chapter 7).

The feasibiltiy and cost-effectiveness of the routine surveillance at the slaughterhouse was modelled for 2012 and presented in the manuscript , Cost and sensitivity of on-farm versus slaughterhouse surveys for prevalence estimation and substantiating freedom from disease: a comparative study using bluetongue, enzootic

bovine leucosis and bovine rhinotracheitis in Switzerland‘, (submitted, chapter 10). As an example of risk factor analysis from data gained at the slaugtherhouse, the prevalence of ESBL in young cattle was analysed and published in , Extended spectrum beta-lactamase producing *Enterobacteriaceae*: occurrence, risk factors for fecal carriage and strain characteristics in the Swiss cattle population younger than 2 years sampled at slaughterhouse level‘, PlosONE, 2013 (Chapter9).

6 Demographic model of the Swiss cattle population for the years 2009-2011 stratified by gender, age and production type

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Abstract

Demographic composition and dynamics of animal and human populations are important determinants for the transmission dynamics of infectious disease and for the effect of infectious disease or environmental disasters on productivity. In many circumstances, demographic data are not available or of poor quality. Since 1999 Switzerland has been recording cattle movements, births, deaths and slaughter in an animal movement database (AMD). The data present in the AMD offers the opportunity for analysing and understanding the dynamic of the Swiss cattle population. A dynamic population model can serve as a building block for future disease transmission models and help policy makers in developing strategies regarding animal health, animal welfare, livestock management and productivity. The Swiss cattle population was therefore modelled using a system of ordinary differential equations. The model was stratified by production type (dairy or beef), age and gender (male and female calves: 0-1 year, heifers and young bulls: 1-2 years, cows and bulls: older than 2 years). The simulation of the Swiss cattle population reflects the observed pattern accurately. Parameters were optimized on the basis of the goodness-of-fit (using the Powell algorithm). The fitted rates were compared with calculated rates from the AMD and differed only marginally. This gives confidence in the fitted rates of parameters that are not directly deductible from the AMD (e.g. the proportion of calves that are moved from the dairy system to fattening plants).

Introduction

Switzerland has been collecting data about cattle including date of birth, date of slaughter, date of death (other than slaughter for consumption) and information regarding movements on a mandatory basis since 1999. The purpose of a national database of animal movements was originally to restore consumer trust during the

BSE crisis by assuring traceability and therefore a better food safety of beef products and to provide a tool for epizootic disease surveillance and control [1,2]. The AMD contains detailed and complete datasets about the Swiss cattle population for several years offering the opportunity to get an insight into the population dynamics. Understanding the demographic of the livestock population in turn provides accurate parameters needed to develop models of disease transmission and helps policy makers in developing strategies regarding animal health, animal welfare and livestock management [3].

Early detection of disease, monitoring of present agents and substantiation of freedom from disease are described as key tasks of modern public veterinary services in order to allow international trade with agricultural goods and to document a good sanitary status of domestic livestock [4–6].

To monitor the health status of the cattle population, the Swiss veterinary authorities invest substantial resources in yearly surveillance programmes that have to meet international standards. One way to maintain the standards while reducing the costs is the application of risk based targeted approaches (e.g. [7]). Other approaches comprise logistical improvements such as better exploiting infrastructures where already a lot of potential information carriers are available e.g. slaughterhouse or milk quality testing laboratories [8]. With the implementation of bulk milk testing in 2010 [9,10] the production type became an important criterion for shaping the sampling strategy of national surveillance programs. As beef and fattening cattle, correspond to one third of the population, they have to be handled separately. The two production types (dairy and beef) do not only differ with respect to purpose but also with respect to management practices. The resulting differences in age distribution and slaughter rates in the two sub populations are of interest for the planning of

stratified surveillance programmes to assure the representativeness of the sample (e.g. for sampling at the slaughterhouse level).

The objective of this study was therefore to create an AMD data driven demographic model that simulates the age and gender specific dynamics of the Swiss cattle population according to the production type. The derived rates describing population dynamics can be used for livestock development planning and associated economic analyses, as a backbone for disease transmission models or for the design of cost-effective disease control and monitoring programmes.

Here we present the first dynamic demographic model of the Swiss cattle population. It is based on over 30 million data points collected in the Swiss animal movement database (AMD) between 2009 and 2011.

Material and Methods

2.1 The Swiss cattle population

The major livestock species in Switzerland is cattle. Although the number of farms decreases, for the years 2009-2011 the number of cattle in Switzerland is stable at roughly 1.6 million animals (table 1). Two thirds of the Swiss cattle industry is dedicated to dairy production. As a consequence, adult dairy cows (older than two years) make the largest demographic segment (figure 1). The average lifespan of a dairy cow in Switzerland is 6.2 years and the average number of calves in a lifetime is 3.7. The oldest cow that died between 2009 and 2011 was 25 years old.

Due to subsidies for ecological and behaviourally sound husbandry and strict animal protection legislation, small holdings with less than hundred animals are still the most common farm type. Over the summer month (May - October) one fourth of the livestock is moved to alpine pastures.

2.2 Data management

The Swiss animal movement database (AMD) contains information on farm level (e.g. location, production type), animal level (e.g. birthdate, gender, and breed), movement records (date, movement type) and stays (i.e. for every animal the start and end date of a stay on any holding is recorded). The data used for the models was an extract from the AMD, containing all recorded movements (25.5 million entries) and stays (15.8 million entries) from January 1999 until January 2012.

Birthdate, date of death (slaughter or natural) and gender are recorded on individual animal level, while the production type is available on farm level. The production type for each animal was consequently determined by the farm it stayed on at the given time step. Calf mortality consisted of notified stillbirths and mortality. As stays on alpine pastures are recorded only since 2008 and the quality of those recordings improved notably in 2009, only data from 2009 to 2011 was used for fitting of the population model.

2.3 The model

The Swiss cattle population was simulated using a system dynamic software [11]. The model is composed of a series of coupled difference equations. Compartments were defined by production type (dairy or beef), age class and gender. Calves were defined as animal being less than one year old, heifer and young bulls as one to two years old and cows and bulls as older than two years. We assumed that cows calve for the first time at the age of two and therefore the category “heifer” doesn’t contribute to births. The beef and dairy system are connected through the transfer of calves from dairy farms to fattening plants, which is represented in the model as “fattening”. The model is represented in figure 2.

The dynamic of the cattle population is simulated by month as time unit. Equations (1) – (12) show the number of animals per compartment (for parameter notation see table 2 and 3).

To represent the seasonal fluctuations in the number of births and death calves, we used a sinusodial-function with amplitude (a), phase (φ) and average (μ) as parameters to fit (equations (13)-(20)). The frequency (ω) was set to $\frac{2\pi}{12}$.

$$\frac{dX_{DF}(t)}{dt} = b_{XDF}(t) * Z_{DF} - (m_{XDF}(t) + s_{XDF} + f_{XDF} + tr_{XDF}) * X_{DF} \quad 0$$

$$\frac{dX_{DM}(t)}{dt} = b_{XDM}(t) * Z_{DF} - (m_{XDM}(t) + s_{XDM} + f_{XDM} + tr_{XDM}) * X_{DM} \quad 0$$

$$\frac{dX_{BF}(t)}{dt} = b_{XBF}(t) * Z_{BF} + f_{XDF} * X_{DF} - (m_{XBF}(t) + s_{XBF} + tr_{XBF}) * X_{BF} \quad 0$$

$$\frac{dX_{BM}(t)}{dt} = b_{XBM}(t) * Z_{BF} + f_{XDM} * X_{DM} - (m_{XBM}(t) + s_{XBM} + tr_{XBM}) * X_{BM} \quad 0$$

$$\frac{dY_{DF}(t)}{dt} = tr_{XDF} * X_{DF} - (m_{YDF} + s_{YDF} + tr_{YDF}) * Y_{DF} \quad 0$$

$$\frac{dY_{DM}(t)}{dt} = tr_{XDM} * X_{DM} - (m_{YDM} + s_{YDM} + tr_{YDM}) * Y_{DM} \quad 0$$

$$\frac{dY_{BF}(t)}{dt} = tr_{XBF} * X_{BF} - (m_{YBF} + s_{YBF} + tr_{YBF}) * Y_{BF} \quad 0$$

$$\frac{dY_{BM}(t)}{dt} = tr_{XBM} * X_{DF} - (m_{YBM} + s_{YBM} + tr_{YBM}) * Y_{BM} \quad 0$$

$$\frac{dZ_{DF}(t)}{dt} = tr_{YDF} * Y_{DF} - (m_{ZDF} + s_{ZDF}) * Z_{DF} \quad 0$$

$$\frac{dZ_{DM}(t)}{dt} = tr_{YDM} * Y_{DM} - (m_{ZDM} + s_{ZDM}) * Z_{DM} \quad 0$$

$$\frac{dZ_{BF}(t)}{dt} = tr_{YBF} * Y_{BF} - (m_{ZBF} + s_{ZBF}) * Z_{BF} \quad 0$$

$$\frac{dZ_{BM}(t)}{dt} = tr_{YBM} * Y_{BM} - (m_{ZBM} + s_{ZBM}) * Z_{BM} \quad 0$$

$$b_{XDF}(t) = \mu_{1XDF} + a_{1XDF} * \sin(t * \omega + \varphi_{1XDF}) \quad 0$$

$$m_{XDF}(t) = \mu_{2XDF} + a_{2XDF} * \sin(t * \omega + \varphi_{2XDF}) \quad 0$$

$$b_{XDM}(t) = \mu_{1XDM} + a_{1XDM} * \sin(t * \omega + \varphi_{1XDM}) \quad 0$$

$$m_{XDM}(t) = \mu_{2XDM} + a_{2XDM} * \sin(t * \omega + \varphi_{2XDM}) \quad 0$$

$$b_{XBF}(t) = \mu_{1XBF} + a_{1XBF} * \sin(t * \omega + \varphi_{1XBF}) \quad 0$$

$$m_{XBF}(t) = \mu_{2XBF} + a_{2XBF} * \sin(t * \omega + \varphi_{2XBF}) \quad 0$$

$$b_{XBM}(t) = \mu_{1XBM} + a_{1XBM} * \sin(t * \omega + \varphi_{1XBM}) \quad 0$$

$$m_{XBM}(t) = \mu_{2XBM} + a_{2XBM} * \sin(t * \omega + \varphi_{2XBM}) \quad 0$$

2.3.1 Model fitting

The number of living animals was extracted at the beginning of each month, number of birth, slaughter and death from the AMD per month, age class, production type and gender from January 2009 to December 2011. This data-set served to optimize the model parameters on the basis of the goodness-of-fit of the nonlinear maximum-likelihood optimization using the Powell algorithm [12]. Parameters were fitted stepwise, adding a variable at every step to the payoff values, using the outcome rates from the previous step as initial search point (maximum and minimum values set to +/- 10%).

2.3.2 Comparison of calculated and fitted rates

Birth, slaughter and mortality rates were calculated from the AMD data and compared to the fitted values from the model. Average birth rates were calculated as number of calves per month and category divided by the number of cows on the first of the

months of the according production type and averaged over the 3 years period. Mortality and slaughter rates were calculated as number of death or slaughtered animals per month divided by the number of animals of the same age category and production type on the first of the month and averaged over the 3 years period. Model and empirical estimates were correlated in R [13].

2.3.3 Sensitivity analysis

The model was rebuilt with the statistical software R. To assess the sensitivity of the model, each parameter was varied separately using a range from -10% to +10% of the fitted value from the Vensim model (baseline), divided in 100 steps. For each value, the resulting absolute change in total numbers of animals compared to the baseline was represented graphically (Figures S1-S10, supplementary material).

Results

In table 4 the fitted parameter values from the demographic model are shown. The model allowed the calculation of parameters that are not directly deductible from the AMD (transition rates and fattening rates).

By introducing parameters (amplitude and phase, table 5) to describe calf mortality and birth rates as trigonometric functions, the seasonal dynamic of changes in the population can be described more accurately than with the corresponding linear parameters deducted from the monthly extracts of the AMD (figure 3).

The correlation of the empirical parameters from the AMD and the fitted values gives a correlation coefficient of 0.994. The good fit of the model to the empirical data is also illustrated in figure 4.

As expected, the beef and dairy sector show differences in the demographic composition. While the proportions of young female animals are comparable (18.5 %

dairy female calves, 17.8 % beef female calves 14.2 % dairy heifers and 14.6 % beef heifers), dairy cows account for around 56.7% of the dairy population while beef cows account for 35.5% of the beef population. For male animals the differences are even more noticeable: beef male calves, young bulls and bulls make 26.5%, 4.6% and 1.1% of the beef population compared to 9.6%, 1.0% and 0.2% for dairy male calves, dairy young bulls and dairy bulls respectively (all proportions are means over the 36 month of data analysis).

As import and export of live cattle are negligible for Switzerland (6'787 imported animals from 2009 to 2011 and 3'318 exported animals over the same period), the beef population is maintained to a considerable extend by calves from the dairy industry. Almost every month more dairy calves are transferred to fattening plants (i.e. to from the dairy to the beef industry) than were born within the beef industry (figure 5).

The number of slaughtered animals does not show a clear seasonal pattern (AMD data, figure 6) and the slaughter rate in the model is linear.

The sensitivity analysis shows, that the dairy female calf birth average and the dairy cow slaughter rate have the biggest influence on the total population with a change in animal numbers bigger than 50'000 after 3 years of simulation (figures S1-S10, supplementary material).

Discussion

4.1 The Swiss cattle population

The composition of the Swiss cattle population accentuates that the milk industry dominates the domestic production and shapes the population dynamic. Adult dairy cows account for over 40% of all animals (figure 4). The importance of dairy female animals for the total population is reflected in the high sensitivity of the beef

population to changes in the dairy cow slaughter rate and the dairy female birth rate (figures S2, S6, supplementary material). The irregular slaughter pattern indicates that the farmers keep the population constant by management decisions.

The higher monthly average mortality of dairy male calves compared to their contemporaries (0.0255 compared to 0.0094 (XDF), 0.0074 (XBM) and 0.0059 (XBF)) is in line with findings of other authors. [14] and [15] found higher mortality rates in dairy breeds than in beef breeds and higher mortality rates in male calves than female calves. As they all defined calves as maximum 180 days of age, the broader categories in our model might explain why dairy male calves differ as much from the others as the effect of early perinatal mortality with higher risk of dystocia for male calves [14] is combined with management decisions, i.e. less care for the economically relatively uninteresting male dairy calves [15]. As we also determined the production type on farm level and not according to the breed as in the above mentioned studies, effects of management decisions on the calve mortality might be even more manifest.

When deducting yearly rates roughly by multiplying the monthly age transition rates by 12, the difference in the management of beef and dairy animals becomes more obvious: while 82% of female dairy calves reach the next age class, only 25% of dairy male calves live through their first year. For beef calves 86% of the females and 61% of the males reach the next age class which reflects the interest of fattening beef breeds for more than 12 month. The most valued group of animals, dairy heifers, reach adulthood in 96% of the cases while more beef heifers are slaughtered and only 74% get two years old.

4.2 Model assumptions

In high productive agriculture systems of the developed world the population dynamics of livestock is controlled by the farmer and depends on policy and economics rather than on resource limitation or other external factors e.g. [16]. Bleul [14] states, that 80 % of Swiss cows are inseminated artificially. For this reason we did not consider a resource constraint i.e. a carrying capacity in our model. The results may be of use for countries in similar economic situation but with less complete records but are to be applied carefully to cattle population that live under more resource dependent natural conditions.

The difference in the birth rates of dairy female and male calves in the model is an artefact presumably due to the difference in the dynamic of the two compartments. Dairy female calves are the most important segment to maintain the population which makes the model sensitive to any change in dairy female calf births. A conservative simulation gives a more stable overall result.

As alpine pastures usually use the gained milk directly for cheese production and it enters therefore not in commerce or they have young stock not yet lactating, they are mostly in the beef category regardless the provenience of the cattle. Therefore the data was corrected over the summer months, using the production type of the farm of origin from the movement records to alpine pastures. The visible seasonal bumps in beef heifers in figure 4 show, that the correction is imperfect due to an incomplete registration of the movements from and to alpine pastures. Since 2012 these are mandatory and improvement of the data quality can be expected.

To integrate the seasonality of birth and mortality in calves, we assumed a sinusoidal pattern and did not investigate other functions.

4.3 Future applications of the model

This is the first dynamic population model for Swiss cattle. As the data source is the complete record of the cattle population, a very good fit could be expected. Nonetheless the fitted population parameters allow a close to reality simulation of the population for future development planning scenario analysis, serve as a backbone to disease transmission models and for the simulation of disease surveillance and control (e.g. [17]).

The fitted population parameters allow building age and sex structured transmission models to simulate disease dynamics with different prevalences in different age classes (e.g. infectious bovine rhinotracheitis IBR, Brucellosis).

Furthermore the transmission rates of different age and production type categories to the slaughterhouse give precise information, which proportions of populations and subpopulations would be basically available for testing at the slaughterhouse in which time period. The slaughterhouse is a very convenient spot for sampling, because it allows taking samples from many animals from different farms of origin within a short time period. Furthermore, there are diseases such as bovine spongiform encephalopathy (BSE) that can only be diagnosed in tissue matrices accessible at slaughter, e.g. brainstem.

As the outcome parameters in the model are calculated for the dairy and beef sector separately, surveillance systems with different components for the different production types can be simulated (e.g. IBR, Brucellosis). For example the efficacy of combining bulk tank milk sampling with slaughterhouse or on farm sampling can be evaluated. As the transfer from calves from the dairy sector to the beef sector is included, the model allows a realistic simulation of disease transmission in the overall population and of the effect of different surveillance strategies on the system sensitivity for different production types.

The fitted population parameters can also be interpreted as baseline parameters for the healthy Swiss cattle population. As seasonal effects are included in the parameter fitting, they can be used to search for aberrations in present data (e.g. increased mortality) to detect health events in an early stage.

In the healthy population most female calves are kept to restock the dairy population, as can be inferred from the relatively low transmission rates of female dairy calves to slaughter. If that segment is affected by an epidemic leading to increased abortions, calf mortality or decreased fertility, consequences on population structure and management are to be expected. Achievement of breeding objectives might be delayed or even out of reach. Impacts on the milk and meat markets are to be expected. The impact on population structure such as decrease of adult dairy cows in the slaughter population can be estimated by model derived transmission factors.

Conclusions

The Swiss animal movement database is a reliable source of information about the Swiss cattle population and can provide stakeholders and decision makers with important knowledge without expensive and laborious field work. The presented demographic model allows a simulation of Swiss cattle production and economics under different policy scenarios and can be used as the demographic backbone for disease transmission models.

References

1. Golan E, Krissoff B, Kuchler F, Calvin L, Nelson K, et al. (2004) Traceability in the U . S . Food Supply : Economic Theory and Industry Studies. Agric Econ Rep 830.

2. Lüdi F (2004) Demographie, räumliche Verteilung und Dynamik der schweizerischen Rindviehpopulation in den Jahren 2002 und 2003 Bern.

3. O'Connor J, More S, Griffin J, O'Leary E (2009) Modelling the demographics of the Irish cattle population. Prev Vet Med 89: 249–254. Available: http://www.ncbi.nlm.nih.gov/pubmed/19327855. Accessed 13 June 2012.

4. Anonymous (2002) Agreement between the European Community and the Swiss Confederation on trade in agricultural products.

5. Anonymous (1995) Tierseuchenverordnung, SR 916.401. Bern. Available: http://www.admin.ch/opc/de/classified-compilation/19950206/index.html.

6. WTO (n.d.) The WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). Available: http://www.wto.org/english/tratop_e/sps_e/spsagr_e.htm.

7. Stärk K, Regula G, Hernandez J, Knopf L, Fuchs K, et al. (2006) Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health : Review of current approaches. BMC Health Serv Res 6:20. doi:10.1186/1472-6963-6-20.

8. Hadorn D, Racloz V, Schwermer H, Stärk K (2009) Establishing a cost-effective national surveillance system for Bluetongue using scenario tree modelling. Vet Res 40:57. doi:10.1051/vetres/2009040.

9. Reber A, Reist M, Schwermer H (2012) Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhino- tracheitis and bovine enzootic leucosis in Switzerland. SAT 154: 189–197. doi:10.1024/0036-7281/.

10. Reist M, Jemmi T, Stärk K (2012) Policy-driven development of cost-effective, risk-based surveillance strategies. Prev Vet Med 105: 176–184. Available: http://www.ncbi.nlm.nih.gov/pubmed/22265642. Accessed 22 September 2014.

11. Ventana Systems I (2008) Vensim®: Simulator for Business.

12. Press W, Flannery B, Teukolsky S, Viatour P (1991) Numerical Recipies in C. Cambridge.

13. R Development Core Team (2008) R: A language and environment for statistical computing. Available: http://www.r-project.org.

14. Bleul U (2011) Risk factors and rates of perinatal and postnatal mortality in cattle in Switzerland. Livest Sci 135: 257–264. Available: http://linkinghub.elsevier.com/retrieve/pii/S1871141310004142. Accessed 21 August 2014.

15. Perrin J, Ducrot C, Vinard J, Hendrikx P, Calavas D (2011) Analyse de la mortalité bovine en France de 2003 à 2009. INRA Prod Anim 24: 235–244.

16. Rosen S, Murphy K, Scheinkman J (2012) Cattle Cycles. J Polit Econ 102: 468–492.

17. Riley S (2007) Large-scale spatial-transmission models of infectious disease. Science 316: 1298–1301. Available: http://www.ncbi.nlm.nih.gov/pubmed/17540894. Accessed 11 July 2014.

Tables

Table 1: The Swiss cattle population 2009-2011. Numbers are extracted from the Swiss animal movement database (AMD)..

Year	No of farms	No of cattle (January 1th)	No of dairy cows (January 1th)	No of slaughtered animals	No of births
2009	42‘966	1‘608‘062	675‘285	647‘715	721‘810
2010	42‘233	1‘610‘277	671‘874	648‘313	719‘004
2011	41‘465	1‘612‘230	676‘253	653‘754	718‘697

Table 2: Nomenclature for subscripts in Equations 1-12.

	Description	type
X	Calves	age class
Y	Subadults	age class
Z	Adults	age class
D	Dairy	production type
B	Beef	production type
F	Female	gender
M	Male	gender

Table 3: Compartments and parameters in Equations 1-12.

	Description	Unit
X	No of calves	Animals
Y	No of subadults	Animals
Z	No of adults	Animals
s	slaughter rate	$month^{-1}$
m	mortality rate	$month^{-1}$
b	birth rate	$month^{-1}$
tr	transition rate	$month^{-1}$
f	fattening rate	$month^{-1}$
μ	Average	$month^{-1}$
a	Amplitude	$month^{-1}$
ω	Frequency	$month^{-1}$
φ	Phase	Dimensionless

Table 4: Monthly population parameters for the Swiss cattle population. D: dairy; B: beef; F: female; M: male; X: calf, Y: subadult, Z: adult. Small letters indicate rates (s: slaughter, m: mortality, f: fattening, tr: transition to next age class). μ1: average birth rate; μ2: average mortality rate;

			Dairy			Beef	
			Month^{-1}	95%-CI		Month^{-1}	95%-CI
slaughter rates	Female calf	s_{XDF}	0.0197	[0.0192, 0.0201]	s_{XBF}	0.0396	[0.0389, 0.0403]
	Heifer	s_{YDF}	0.0065	[0.0062, 0.0069]	s_{YBF}	0.0261	[0.0253, 0.0269]
	Cow	s_{ZDF}	0.0190	[0.0189, 0.0191]	s_{ZBF}	0.0233	[0.0231, 0.0235]
	Male calf	s_{XDM}	0.1123	[0.1103, 0.1144]	s_{XBM}	0.0638	[0.0631, 0.0645]
	Young bull	s_{YDM}	0.1702	[0.1658, 0.1748]	s_{YBM}	0.2834	[0.2768, 0.2902]
	Bull	s_{ZDM}	0.1113	[0.1072, 0.1156]	s_{ZBM}	0.0606	[0.0590, 0.0623]
mortality rates	Female calf	μ_{2XDF}	0.0094	[0.0089, 0.0098]	μ_{2XBF}	0.0059	[0.0055, 0.0062]
	Heifer	m_{YDF}	0.0007	[0.0006, 0.0007]	m_{YBF}	0.0008	[0.0007, 0.0009]
	Cow	m_{ZDF}	0.0013	[0.0012, 0.0013]	m_{ZBF}	0.0013	[0.0013, 0.0014]
	Male calf	μ_{2XDM}	0.0255	[0.0241, 0.0269]	μ_{2XBM}	0.0074	[0.0071, 0.0078]
	Young bull	m_{YDM}	0.0017	[0.0015, 0.0019]	m_{YBM}	0.0017	[0.0015, 0.0019]
	Bull	m_{ZDM}	0.0022	[0.0016, 0.0028]	m_{ZBM}	0.0026	[0.0021, 0.0031]
transition rates	Female calf	tr_{XDF}	0.0684	[0.0678, 0.0689]	tr_{XBF}	0.0718	[0.0710, 0.0725]
	Heifer	tr_{YDF}	0.0804	[0.0797, 0.0812]	tr_{YBF}	0.0615	[0.0607, 0.0624]
	Male calf	tr_{XDM}	0.0207	[0.0203, 0.0212]	tr_{XBM}	0.0511	[0.0505, 0.0518]
	Young bull	tr_{YDM}	0.0234	[0.0226, 0.0243]	tr_{YBM}	0.0161	[0.0157, 0.0165]
fattening rates	Female calf	f_{XDF}	0.0172	[0.0170, 0.0175]			
	Male calf	f_{XDM}	0.0731	[0.0722, 0.0740]			
birth rates	Female calf	μ_{1XDF}	0.0374	[0.0373, 0.0376]	μ_{1XBF}	0.0335	[0.0332, 0.0339]
	Male calf	μ_{1XDM}	0.0392	[0.0389, 0.0396]	μ_{1XBM}	0.0352	[0.0347, 0.0357]

Table 5: Values for the amplitudes and phases in the trigonometric functions of the presented Swiss cattle population model. D: dairy; B: beef; F: female; M: male; X: calf, Y: subadult, Z: adult. a 1: amplitude for birth rate; a 2: amplitude for mortality rate; φ_1: phase for birth rate; φ_2: phase for mortality rate;

	Dairy			Beef	
		95%-CI			95%-CI
a_{1XDF}	0.0031	[0.0022, 0.0041]	a_{1XBF}	0.0009	[0, 0.0023]
a_{1XDM}	0.0091	[0.0073, 0.0109]	a_{1XBM}	0.0040	[0.0024, 0.0056]
a_{2XDF}	0.0029	[0.0020, 0.0038]	a_{2XBF}	0.0013	[0.0008, 0.0018]
a_{2XDM}	0.0063	[0.0037, 0.0088]	a_{2XBM}	0.0016	[0.0010, 0.0022]
φ_{1XDF}	1.6799	[1.4046, 1.9574]	φ_{1XBF}	2.9510	[1.1437, 4.7768]
φ_{1XDM}	1.9245	[1.7428, 2.1096]	φ_{1XBM}	2.4772	[2.0699, 2.8935]
φ_{2XDF}	1.6576	[1.3443, 1.9727]	φ_{2XBF}	1.0713	[0.6834, 1.4582]
φ_{2XDM}	1.7900	[1.3820, 2.1969]	φ_{2XBM}	0.9218	[0.5575, 1.2856]

Figures

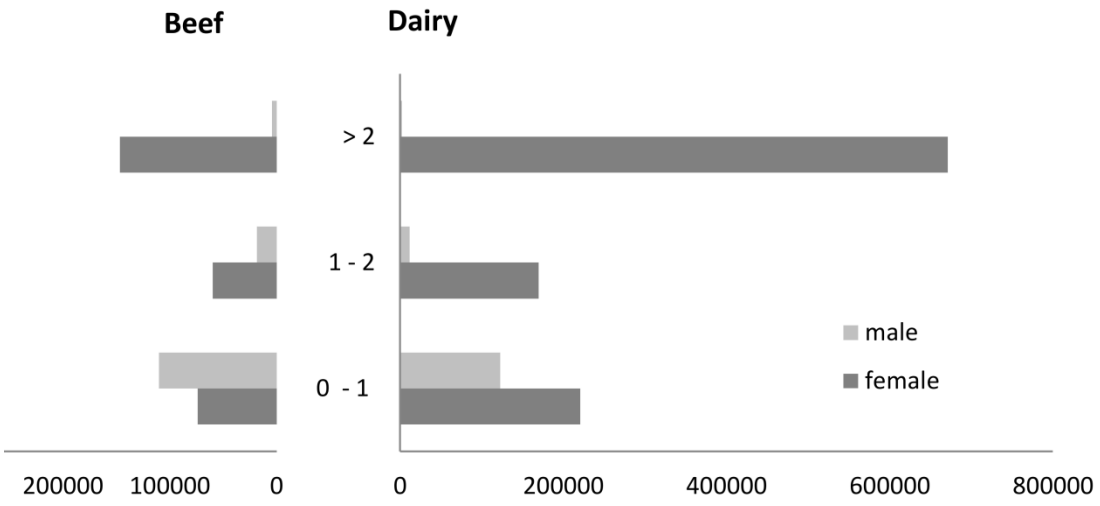

Figure 1: Demographic of the Swiss cattle population per age class and sex in number of animals.

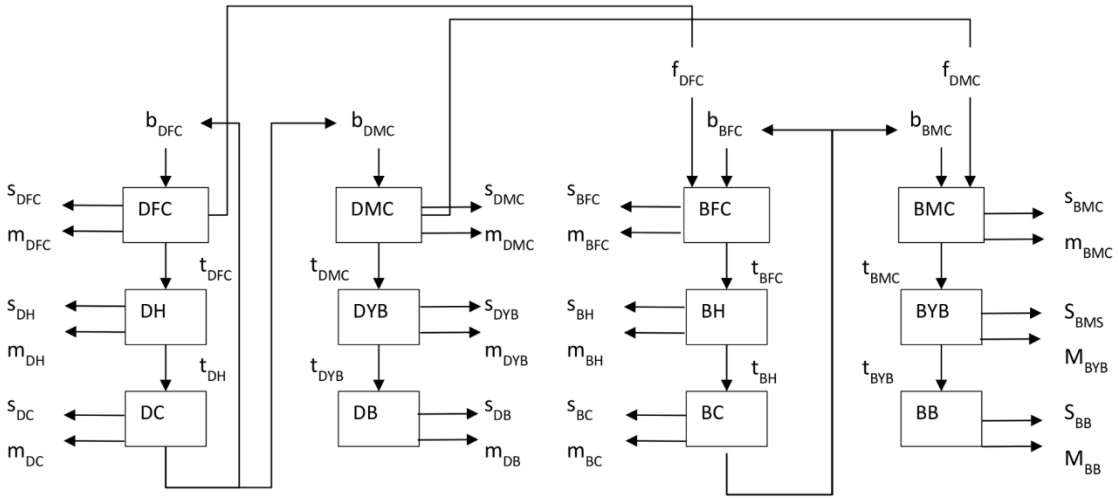

Figure 2: Schematic representation of the Vensim model. Arrows represent flows of animals into or out of a compound, boxes represents numbers of animals at a given time point in a category. s: slaughter; m: mortality; b: birth; tr: transition; f: fattening; D: dairy; B: beef; F: female; M: male; X: calves; Y: subadults; Z: adults.

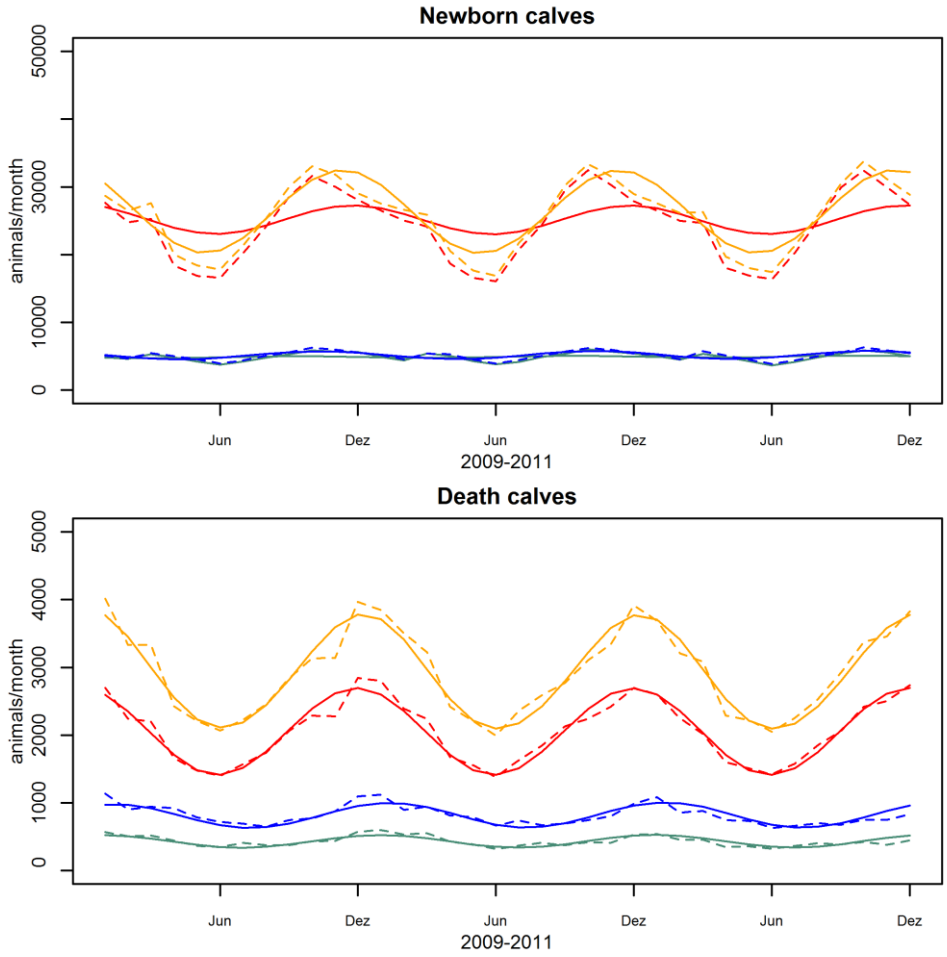

Figure 3: Seasonal pattern of birth and mortality in calves. Solid line: model data, dashed lines: AMD data. Orange: dairy male calf, red: dairy female calf, blue: beef male calf, green: beef female calf.

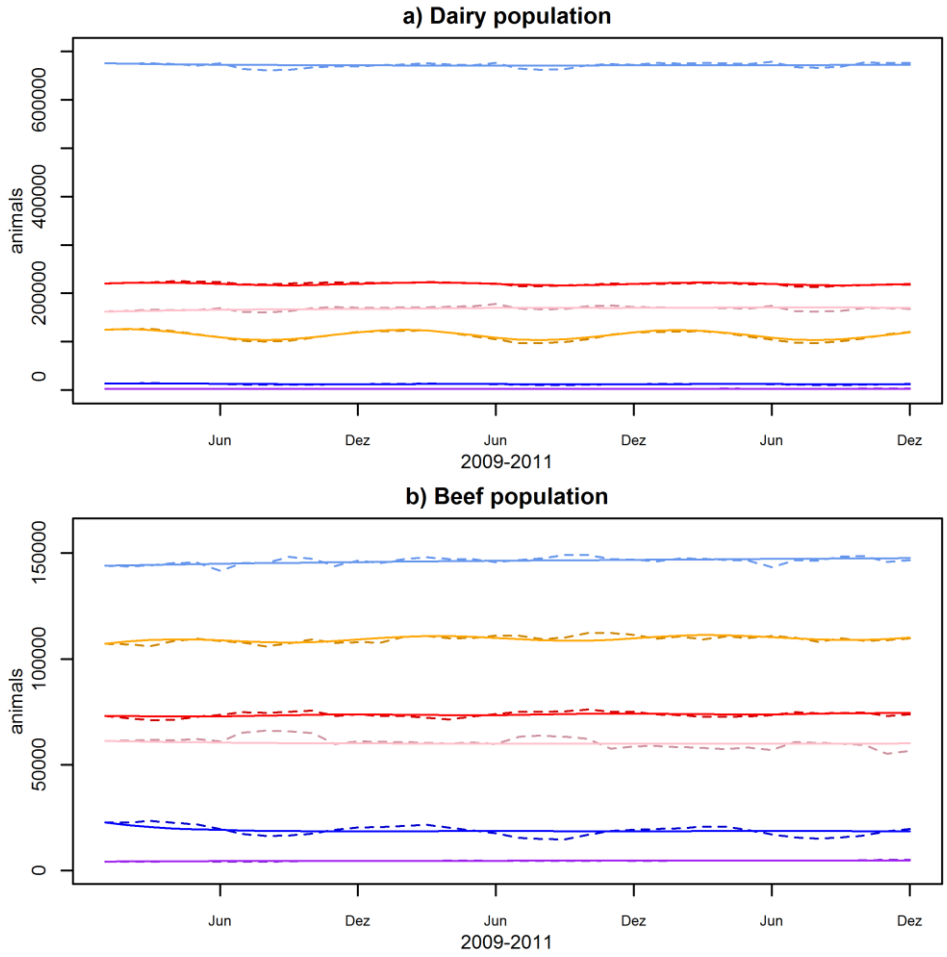

Figure 4: Animal numbers per age category. a) Dairy population. b) Beef population. Solid line: model data, dashed lines: AMD data. Light blue: cow, orange: male calf, red: female calf, pink: heifer, blue: young bull, purple: bull.

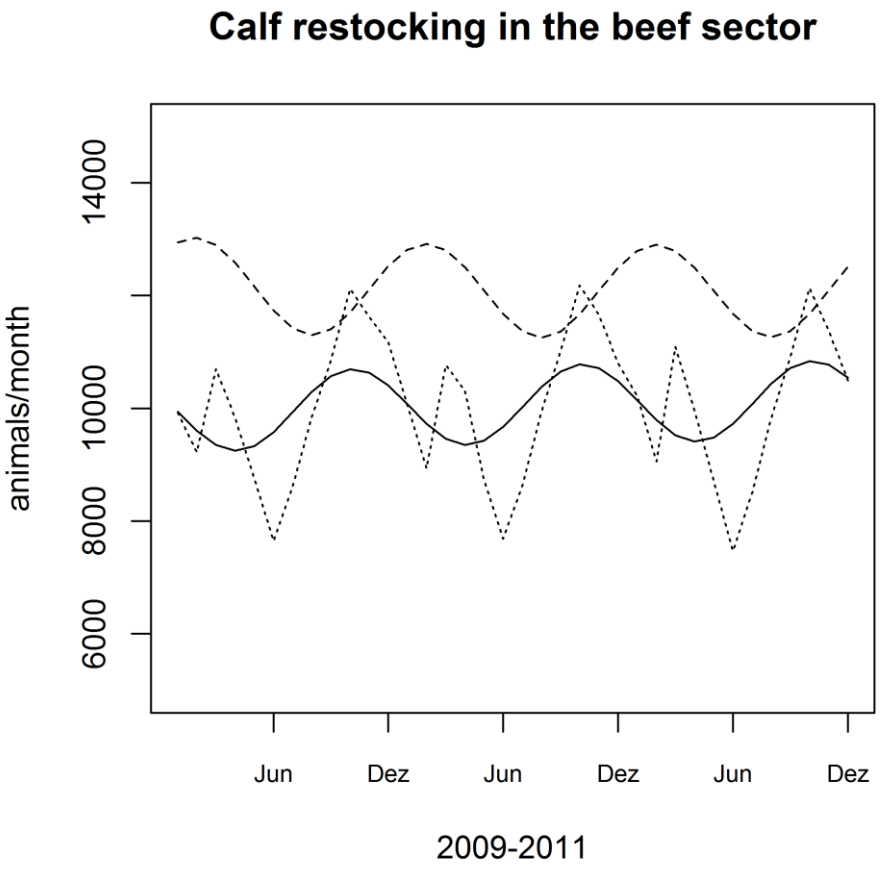

Figure 5: Restocking of calves in the beef sector. Dashed line: dairy calves transferred to fattening plants (VENSIM), solid line:born beef calves (VENSIM), dotted line: born beef calves (AMD)

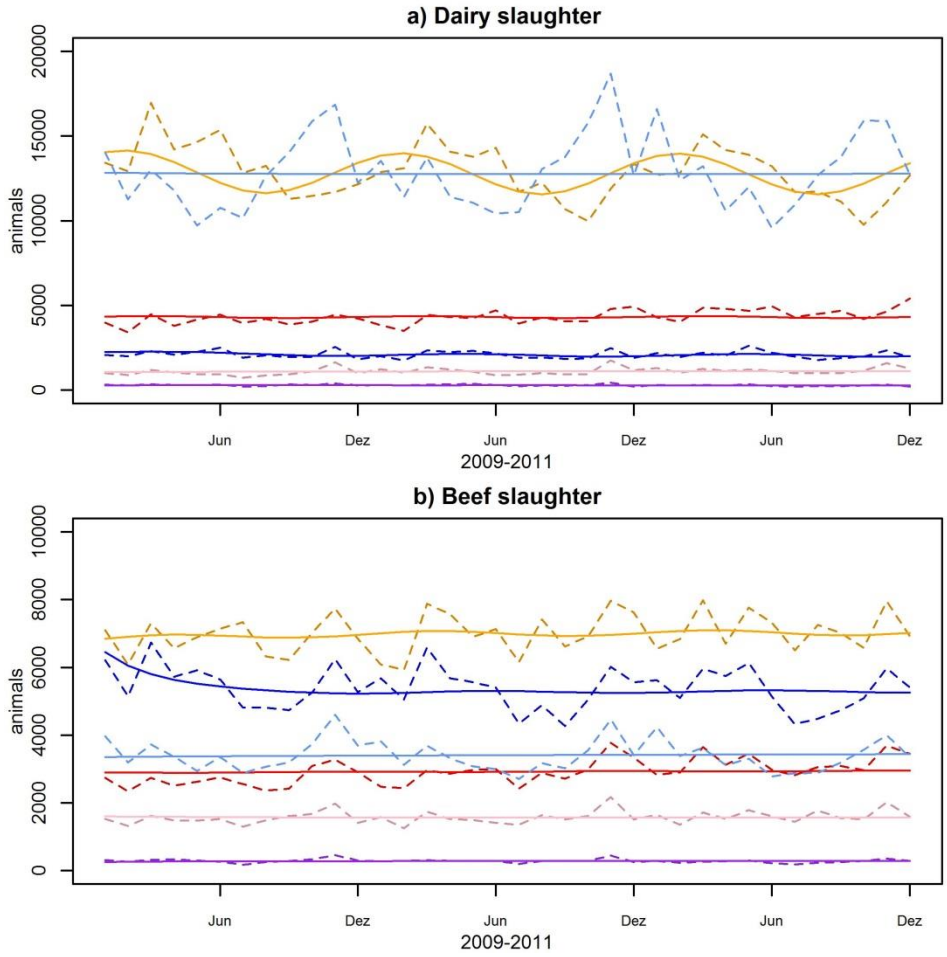

Figure 6: Slaughter numbers per age category. a) Dairy population. b) Beef population. Solid line: model data, dashed lines: AMD data. Light blue: cow, orange: male calf, red: female calf, pink: heifer, blue: young bull, purple: bull.

Supplementary material

Text S1: Sensitivity analysis

The graphical representation of the sensitivity analysis of the demographic model of the Swiss cattle population shows, how the parameters in the model influence the number of animals in the total, the dairy and the beef population respectively. Each parameter was varied separately using a range from -10% to +10% of the fitted value from the Vensim model (baseline), divided in 100 steps.

The figures S1-S4 show the change in numbers of animals using different colour palettes for the change in the dairy population (aquamarine-blue-magenta) and the beef population (violet-turquoise-yellow). Contour lines indicate the absolute change in number of animals (10, 100, 1’000, 5’000, 10'000, 50'000).

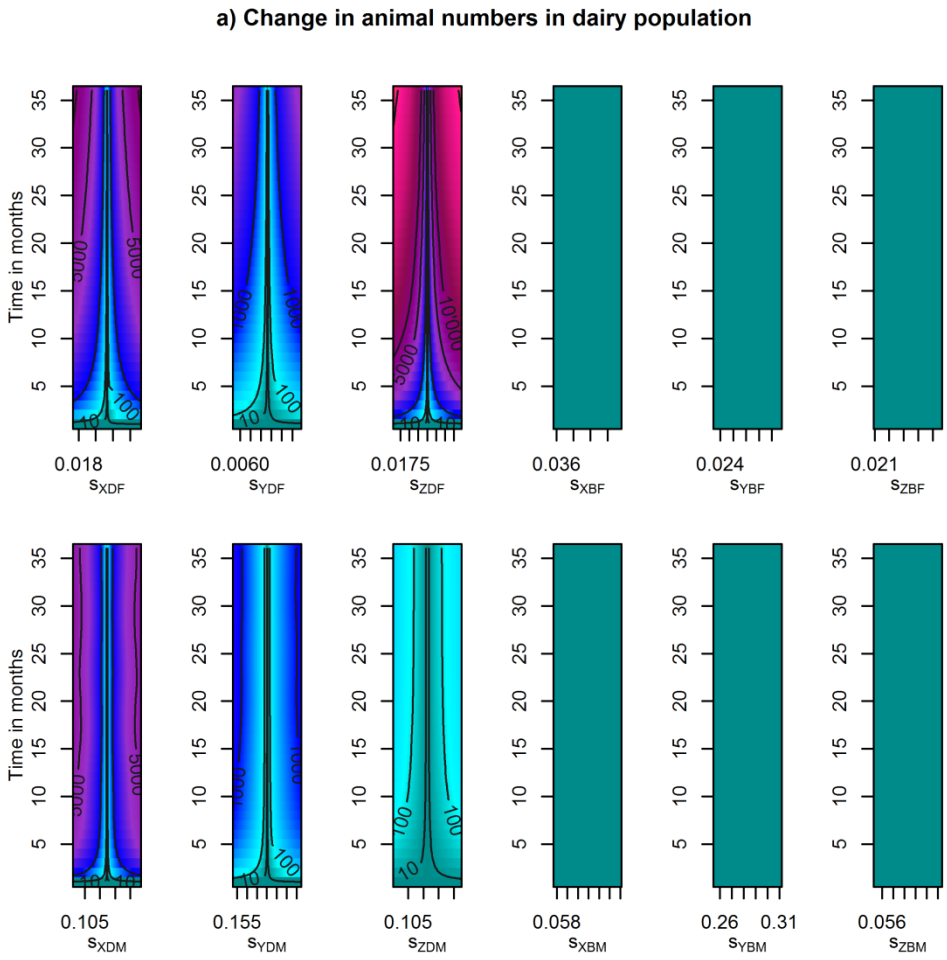

Figure S1: Influence of varying slaughter rates on the number of animals in the dairy population

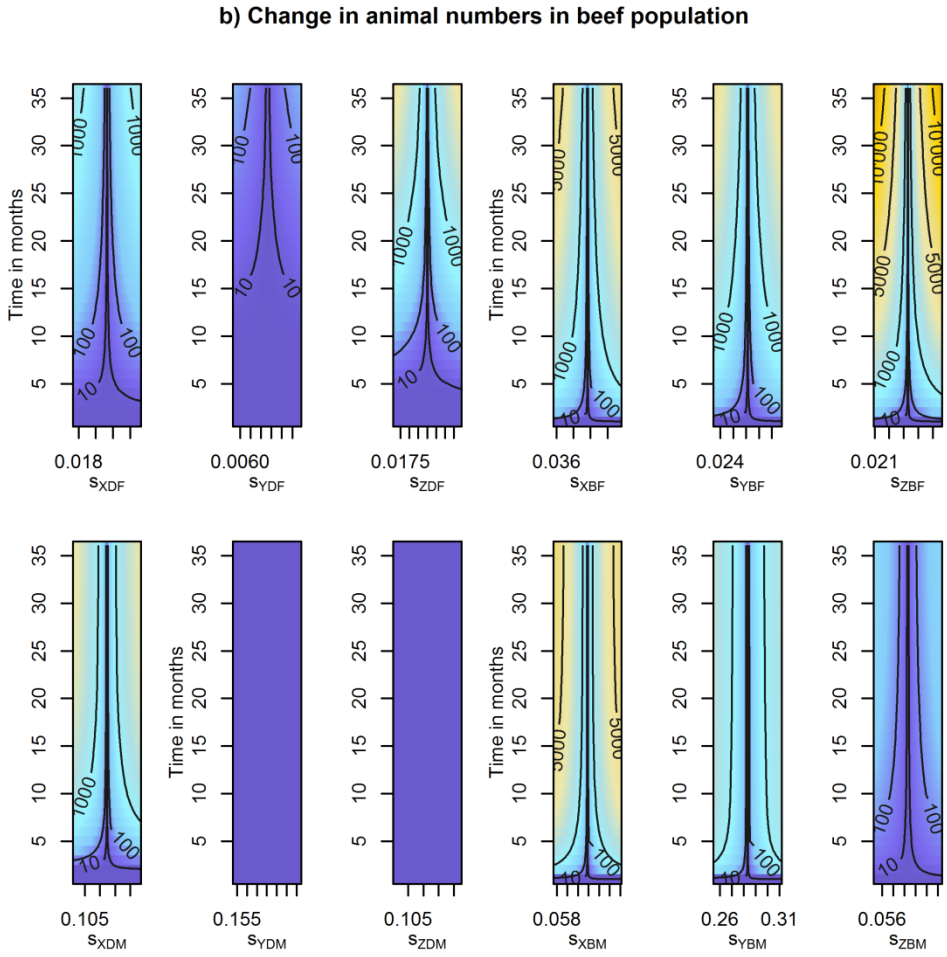

Figure S2: Influence of varying slaughter rates on the number of animals in the beef population

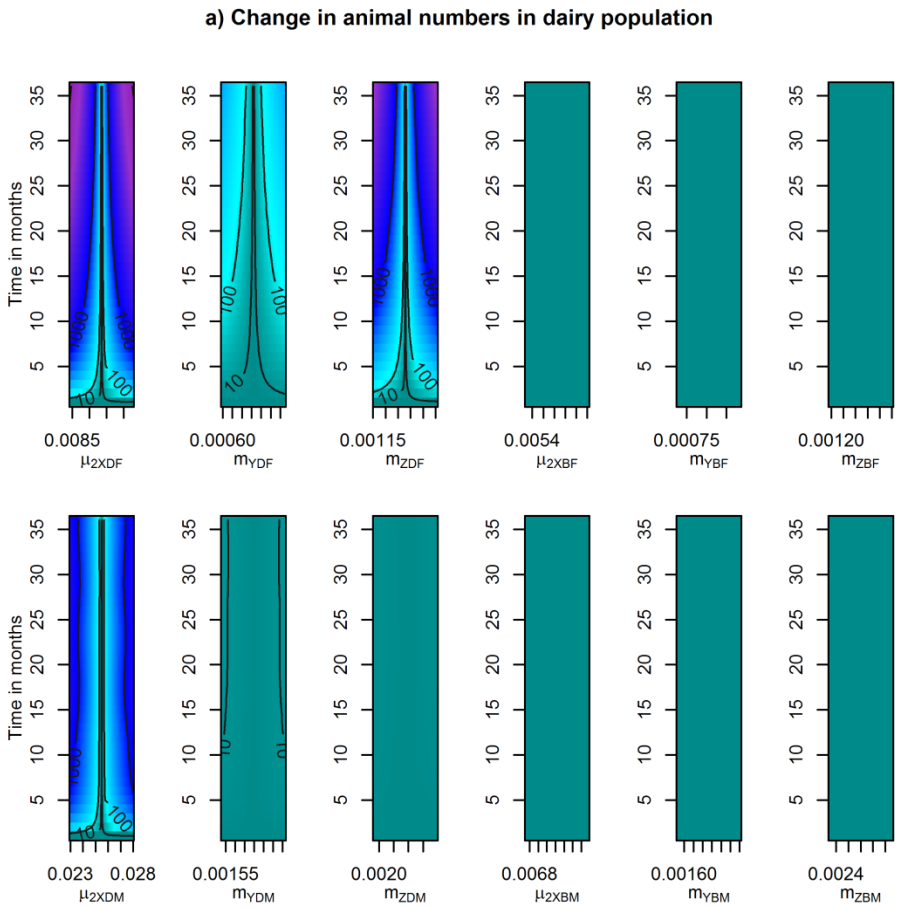

Figure S3: Influence of varying mortality rates on the number of animals in the dairy population.

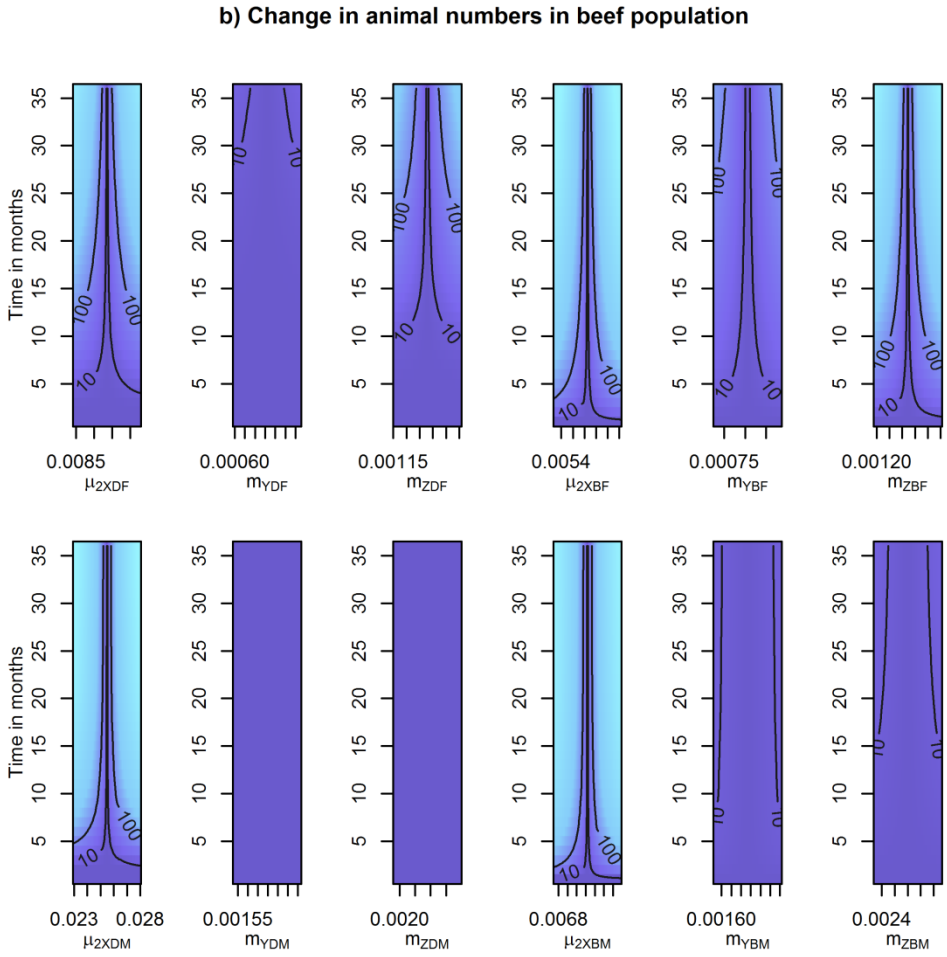

Figure S4: Influence of varying mortality rates on the number of animals in the beef population

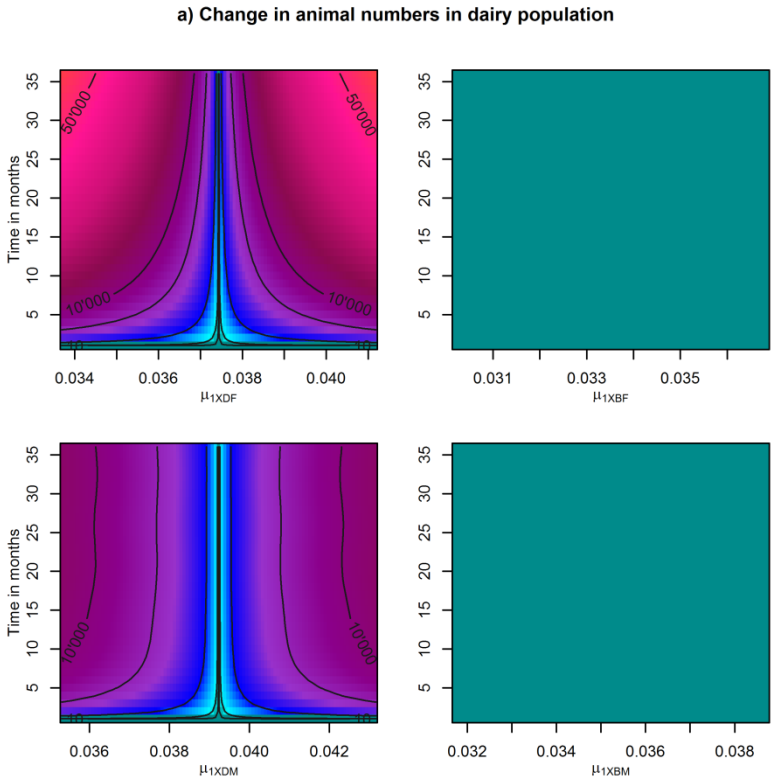

Figure S5: Influence of varying average birth rates on the number of animals in the dairy population.

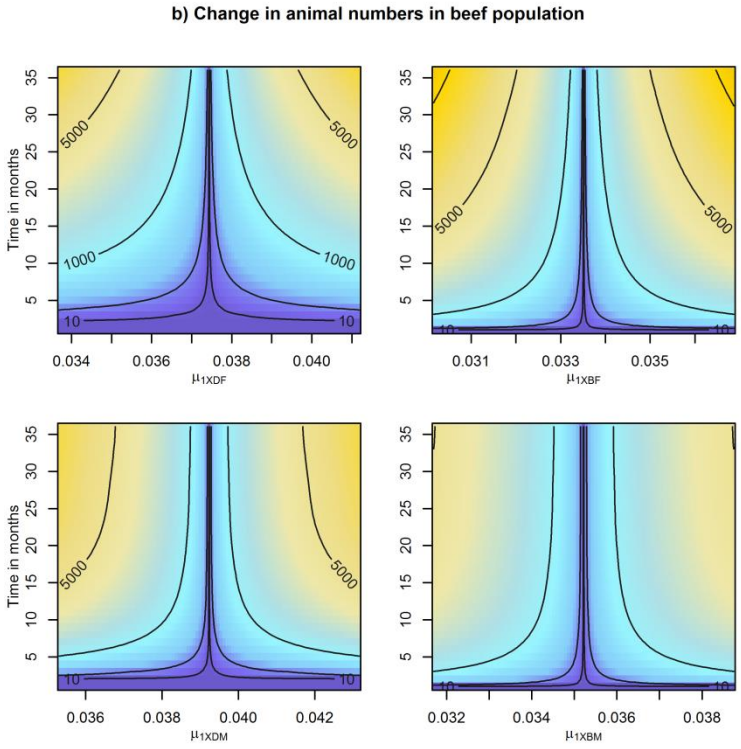

Figure S6: Influence of varying average birth rates on the number of animals in the beef population.

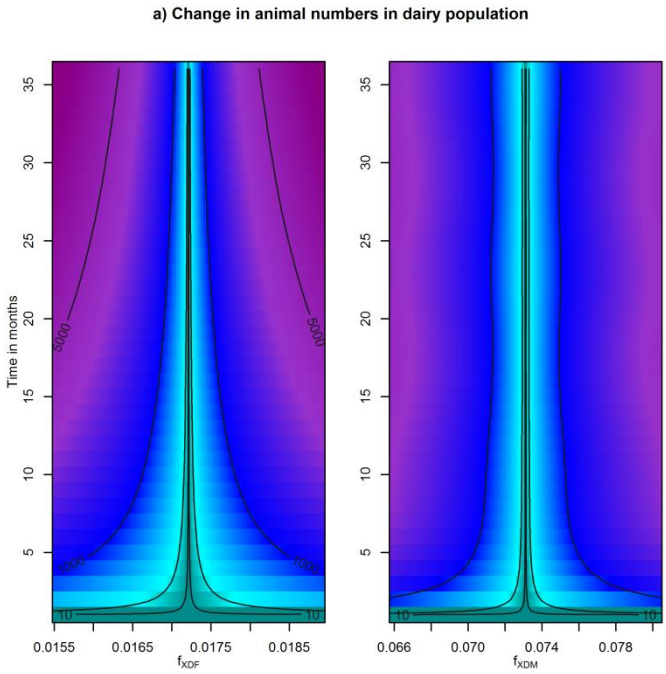

Figure S7: Influence of varying fattening rates (calves transferring from the dairy to the beef sector) on the number of animals in the dairy population.

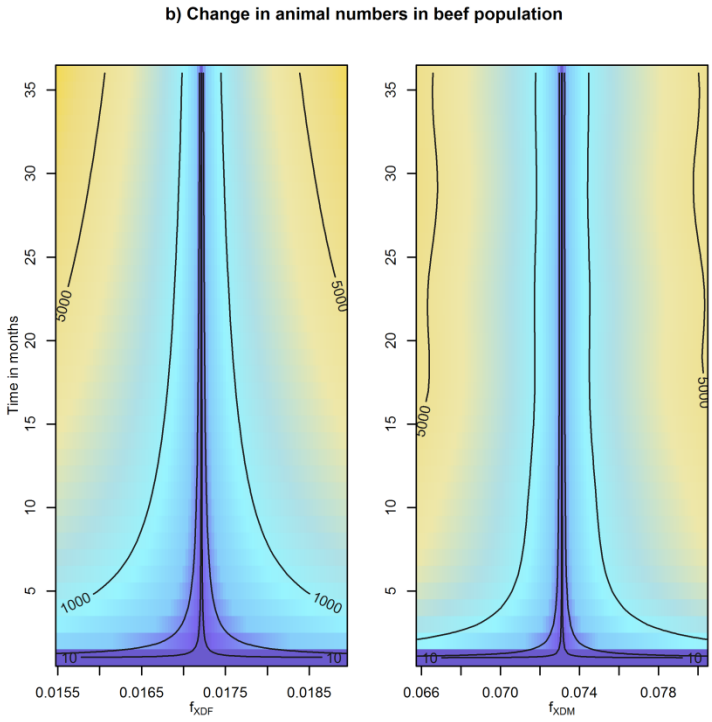

Figure S8: Influence of varying fattening rates (calves transferring from the dairy to the beef sector) on the number of animals in the beef population.

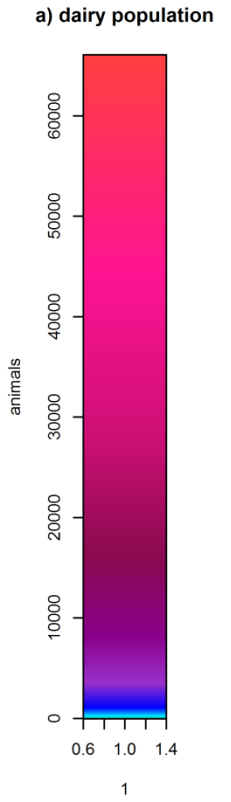

Figure S9: legends for the colour scales for the dairy population.

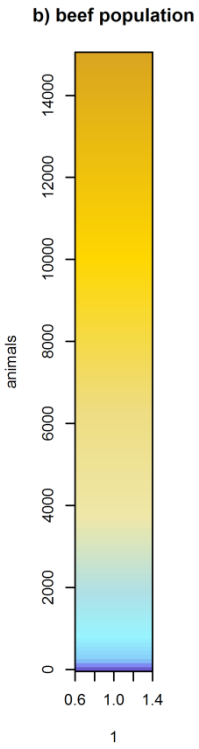

Figure S10: legends for the colour scales for the beef population

7 Evaluation of farm-level parameters derived from animal movements for use in risk-based surveillance programmes of cattle in Switzerland

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Abstract

Background

This study focused on the descriptive analysis of cattle movements and farm-level parameters derived from cattle movements, which are considered to be generically suitable for risk-based surveillance systems in Switzerland for diseases where animal movements constitute an important risk pathway.

Methods

A framework was developed to select farms for surveillance based on a risk score summarizing 5 parameters. The proposed framework was validated using data from the bovine viral diarrhoea (BVD) surveillance programme in 2013.

Results

A cumulative score was calculated per farm, including the following parameters; the maximum monthly ingoing contact chain (in 2012), the average number of animals per incoming movement, use of mixed alpine pastures and the number of weeks in 2012 a farm had movements registered. The final score for the farm depended on the distribution of the parameters. Different cut offs; 50%, 90%, 95% and 99%, were explored. The final scores ranged between 0 and 5. Validation of the scores against results from the BVD surveillance programme 2013 gave promising results for setting the cut off for each of the five selected farm level criteria at the 50th percentile. Restricting testing to farms with a score ≥ 2 would have resulted in the same number of detected BVD positive farms as testing all farms, i.e. the outcome of the 2013 surveillance programme could have been reached with a smaller survey.

Conclusions

The seasonality and time dependency of the activity of single farms in the networks requires a careful assessment of the actual time period included to determine farm level criteria. However, selecting farms in the sample for risk-based surveillance can

be optimized with the proposed scoring system. The system was validated using data from the BVD eradication program. The proposed method is a promising framework for the selection of farms according to the risk of infection based on animal movements.

Keywords: cattle movements, risk score, bovine viral diarrhoea, Animal movement database, risk-based surveillance

Background

Animal movements are an important driver for the spread of contagious diseases [1–4]. Information about animal movements and the resulting contact network are therefore of great value for surveying and controlling animal diseases [5–7].

Over the past years, methods that have been developed for social network analysis in human sciences have also been used to describe and summarize data on animal movements [8]. The network theory describes how entities are connected with each other and patterns formed by these connections. The units of interest are called nodes. The undirected connections between them are called edges, and arcs represent directed connections [9, 10]. These methods were used to analyse disease transmission through human to human contact in the 1990s, especially for HIV/AIDS and other sexually transmitted diseases [11]. In contrast to human sciences, veterinary epidemiology mainly focuses on a collective unit, such as a premise or farm, rather than on the individual animal. The premises are considered as nodes whereas animals moved from one premise to another form the arcs.

Patterns revealed by analysing network structures and metrics can improve the understanding of livestock industry in a country, and result in more effective decision making and control measures in case of disease outbreaks [12, 13]. For targeted surveillance purposes, the number of direct contacts of farms can be used to identify and prioritise premises with an important role in the contact network [7, 14].

Most of the traditional network metrics describe a static network considering all arcs to be permanent. However, in animal movement networks, arcs are only active over a short period of time and therefore, the sequence of movements is important to understand potential disease transmission patterns. Such temporal networks were subject of numerous recent studies [15–17]. A path in a temporal network between two premises exists only if all connecting movements are in a time sequence (see

figure 1). By arranging contacts between premises in a chronological order, the temporal dimension of the network is accounted for. This allows backwards and forward tracing of potentially infected farms in case of an outbreak. To track potentially infected farms from a given source, the infection chain was proposed by Dubé et al. [8]. Nöremark et al. [9] refined this concept by introducing the ingoing contact chain to trace back potential sources of infection. The ingoing contact chain contains all possible paths onto a premise in a given time interval, taking the sequence by which the connecting movements occur into account. The ingoing contacts and corresponding contact chain have been shown to be relevant measures for the probability of disease detection in the final herd of destination [7, 18].

Due to a significant beef and dairy industry, Switzerland invests substantial resources into the surveillance of its main livestock species. Developing methods which reduce cost of surveillance without losing effectiveness is a priority of decision makers [19]. Yearly serological surveillance programmes to substantiate freedom from disease could be optimized by targeting the sampling to farms where disease occurrence is most likely [19–22]. Currently, targeted selection of at-risk farms is utilised in the yearly surveillance programmes to substantiate freedom from infectious bovine rhinotracheitis (IBR), enzootic bovine leucosis (EBL) and bluetongue (BT). The risk factors considered for IBR and EBL in these programmes are the number of cattle moved on farm and the use of transhumance [23].

Additionally, all cattle farms are under surveillance for bovine viral diarrhoea (BVD) in the final stage of the national eradication programme. BVD is an important production disease in cattle, associated with fertility disorders and production loss. Bovine viral diarrhoea virus (BVDV) has a unique capacity to cause persistent infections of

foetuses exposed within the first 150 days of gestation. Persistently infected (PI) calves shed large quantities of virus for life and are primarily responsible for sustaining disease transmission at the population level [24, 25].

For the eradication in Switzerland, dairy farms are tested annually while other cattle farms are tested every third year. Beginning in 2008, every bovine was tested for BVD antigen and positive animals were slaughtered. From 2009 to 2012 all new-born calves were tested for BVD antigen by ear-notch sampling. In 2012, a serological surveillance programme was introduced comprising bulk-tank milk sampling for dairy and on-farm blood-sampling for non-dairy farms [26, 27]. In 2013, the herd-level incidence of BVD (farms with persistently infected animals) was below 0.5% and the comprehensive testing of new born calves was halted. Bovines on farms with any positive result (serological or antigen) are thoroughly tested. In 2014, the herd-level incidence had dropped to 0.12%. As it is well established that BVD is introduced primarily through the movement of persistently infected animals (PI) or cows carrying a PI, farms which receive many animals from many farms are at higher risk to get infected and surveillance should target on such farms [24, 28–30].

Routine surveillance programmes are planned and conducted on a yearly basis. Therefore, any potential farm-based parameters for risk classification should reflect the same time intervals. The current surveillance programmes conducted in Switzerland rely on serological testing.

The shared alpine pastures constitute a risk for disease transmission because of the mixing of different herds over three to five months. The animal contacts occur at watering places or salt licks, providing a pathway for the spread of other diseases [31].

This study focused on identifying farm-level parameters associated with cattle movements, which could be used to classify Swiss farms for targeted surveillance of contagious diseases.

Material and Methods

Cattle movement data from 2012 were used and the resulting networks were described to investigate the seasonality in the cattle network and to better understand the network as a whole.

Several parameters were chosen or developed based on their likely association with the risk of acquiring disease via animal movement. A framework was developed to select farms for surveillance, based on a risk score. The proposed framework was validated using data from the BVD surveillance programme in 2013.

Data

Cattle represent the majority of livestock species in Switzerland with 1.6 Million recorded animals in 2012. Livestock farms are small scale with about 40 bovines per farm. In the summer months (May - October) half of the cattle farms move some animals to the mountains for seasonal, often collective pasturing. In total, about 25% of the Swiss bovines spend the summer month on alpine pastures.

The animal movement database (AMD) is the mandatory, nationwide registry for cattle in Switzerland. It holds records of all premises, individual bovines and movements of bovines between farms. The data is publicly available on the joint portal of the federal office of agriculture (FOAG) and the federal food safety and veterinary office (FVO) [32].

Cattle owners must report all transfers of animals to other premises within 3 working days. To ensure compliance, the completeness of an animal's movement history is a requirement to receive full subsidies for that bovine at slaughter. Reported movements and living stock are also cross-checked with the AMD records during regular official inspections on farm. The transports from farms to slaughterhouses are often conducted by traders that collect animals from different farms and deliver them directly to slaughter.

Using data from the AMD, premises were categorized as follows; farm (41'474), market (189), slaughterhouse (599), alpine pasture (6'451) and clinic (5). The movements between these categories can be in either direction, except that by law there should be no livestock leaving a slaughterhouse. The average herd size was calculated from twelve reverence dates in 2012 (the first day in every month).

For the network analysis, all movements in 2012 were extracted from the AMD. From a total of 907'593 registered movements, 904'351 were complete unique records and included in the analysis. Premises were considered as nodes, and cattle moved between the premises represented the arcs. A movement was defined as 'cattle moved on one day from the premise of origin to the premise of destination'.

Movements and herd composition were investigated using summary statistics. To assess the herd structure over time, the presence or absence of bovines recorded as present at 1st January were subsequently determined on the following reference dates.

Network metrics

As stated in the background section, animal movement networks are temporal networks and network metrics depend on the underlying time interval. To give an

overview over the entire study period, the presented metrics were calculated for the network consisting of cattle movements between the 1st January and 31st December 2012. Additionally, the same metrics were calculated for twelve monthly networks January – December 2012.

In such temporal networks, a path from node A to node B to node C (A is directly connected to B and B is directly connected to C) exists only if the movement from A to B happens before the movement from B to C. Otherwise A and C are disconnected as no animals can move from A to C via B [15]. In the cattle trade network the transfer of bovines from one premise to another happens at a very specific point in time and connections between premises cannot be considered permanent. To account for the temporal nature of the underlying network, only metrics that are applicable in temporal networks are used in this study, i.e. if paths are built within the network, the chronology of the movements must be considered.

On farm level, the in-degree (ID), out-degree (OD), the ingoing contact chain (ICC) and the outgoing contact chain (OCC) were calculated. The ID is defined as the number of individual sources providing animals directly to a specific livestock operation and the OD as the number of individual recipients obtaining animals directly from a specific livestock operation [33].

The OCC, which is sometimes referred to as ‘accessible world’ or ‘output domain’, is the number of premises in contact with a certain premise through movements of animals leaving the premise. The metric captures contacts both through direct movements, as well as indirect contact through further movements, and the sequence of the movements is taken into account [9, 14, 34]. Holme & Saramäiki [15] describe this as the *set of influence* of the node in question, i.e. the set of nodes that can be reached by the node through time respecting paths within the observation

window. The ICC measures all direct and indirect contacts through movements onto a premise. Similar to the OCC, the metric captures contacts both through direct movements, as well as indirect contact through further movements, and the sequence of the movements is taken into account [14]. Holme & Saramäiki [15] describe this as the *source set* of the node in consideration, i.e. the set of nodes that can reach the node through time respecting paths within the observation window. The ID, OD, ICC and OCC are illustrated in figure 1. In a static representation of the same network as in figure 1 b), nodes D and C would be connected via node E. In the temporal network presented, this connection does not exist because the movement from E to C happens earlier than the movement from D to E. The ID and OD however, are calculated the same way as in a static network. The distributions of the ID, OD, ICC and OCC were used to describe trade network on network level.

As a temporal counterpart to the giant strong component (GSC, [9]) the reachability ratio (reR) was included in the analysis. The outgoing reachability ratio (out-reR) measures the fraction of all premises that are included in the OCC's in a certain observation window [35]. The fraction of premises another premise 'is reached by', or the fraction of premises included in the source set, was measured as fraction of premises in the largest ICC's (in-reR). For all distributions, mean, median, maximum and skewness (g_1, see [36]) were reported.

Movements were not weighted for the calculation of the network metrics (i.e. the number of cattle per movement was not considered).

Farm level parameters based on cattle movements

Six movement-related farm level parameters were derived from the AMD data and were assessed for their usefulness in risk-based surveillance.

For surveillance purposes, farms with high numbers of premises in the direct or indirect ingoing contact chains are of interest [7, 37]. Therefore, in the choice of farm level parameters for the risk score we considered only the metrics describing movements onto a farm. We selected the time window for each metric considering two aspects; the annual rhythm of the surveillance programmes, which defines the period for which we need information, and the epidemiological relevance. For the ID, a year was considered a reasonable time period, capturing a full seasonal cycle (ID_y). However, for the ICC, a time period of one year would result in the inclusion of hubs like alpine pastures, markets and annual fairs in the chains, eventually connecting almost all premises. Therefore, the largest ICC (ICC_{max}) among the 12 monthly networks for each farm was chosen. The maximum of these 12 values was chosen to capture farms with many potential sources of infection in the year considered, while limiting the observation period to a more reasonable time period for the spread of an undetected infectious disease event.

While ICC_{max} and ID_y are both indicators for the number of premises a farm can get infected animals from, the average number of animals per incoming movement and farm (average animals per movement, avAN) was included as a parameter to account for the increasing probability of receiving an infected or sero-positive bovine when more animals are moved on to the farm. To measure the importance of a farm in the network, the fraction of times a farm is on the shortest temporal path between two premises of all existing shortest temporal paths (number shortest paths, NS) was calculated for the monthly networks. This can be seen as the temporal network analogy to the betweenness in a static network, i.e. the frequency a livestock operation is on the shortest path between pairs of operations in a static network [38].

Finally, accounting for the dynamics of temporal networks, the number of weeks a farm was active and had movements registered was included as a parameter (active weeks, AW). Whether or not a farm sent animals to, and received animals from, a shared alpine pasture was included as a binary parameter (movement to alpine pasture, MA). Table 1 gives an overview of the selected network metrics and constructed parameters that were considered for the score.

Measurements of association and risk score

For every farm, the farm level parameters (except MA) were binary scored (1/0) according to their position in the distribution of the values for all farms for four different thresholds (i.e. above or below the threshold). Thresholds were set at the 50th, 90th, 95th and 99th percentiles. The association among the selected farm level parameters was investigated using Spearman rank correlation. The correlation between herd size and the selected parameters was also investigated. The NS was then excluded because it was strongly correlated with the ICC_{max} (see discussion for the reasons for this decision).

The score for every parameter in the final set was determined for each farm, at the different thresholds.

Finally, the scores were summarised to give the 'network based' risk score for every farm. The score ranged from 0-5.

Validation

For the validation of the scoring system, data from the serological surveillance for BVD in 2013 was used.

The two main components of the BVD surveillance programme were considered; bulk tank milk sampling twice a year for dairy farms and one spot test (blood sample of a group of young animals) for non-dairy farms. Small farms with less than 10 bovines were in a different surveillance scheme and were thus excluded from the dataset. All farms free from BVD at the beginning of 2013 and farms with a positive surveillance result in 2013 were included. The status “BVD free” for farms at the beginning of 2013 is of high certainty, as the cattle population was tested comprehensively for 6 years. For the validation, negative farms are farms with no evidence of BVD infection during the eradication programme including 2013 (n= 1’561), whereas positive farms are those with a PI in 2013 (n=29).

The presence of PI animals was either the result of an ongoing infection or by a newly introduced infection. Only PIs associated with new infections were seen as relevant to validate the network based risk score. The most likely source for new infections are movements of PIs or dams carrying a PI onto a free farm.

The risk score of the constantly BVD free farms in the BVD surveillance programme 2013 was compared with the score for newly infected farms.

The sensitivity (Se) and specificity (Sp) for detecting the farms with a PI using the proposed risk score was calculated using the following formulas: $Se = \frac{TP}{(TP + FN)}$, $Sp = \frac{TN}{(TN + FP)}$ where TN are the number of true negative, TP the true positive, FN the false negative and FP the false positive farms [39]. Scores resulting from all four thresholds were assessed.

Software

Data analysis was conducted using R (version 3.1.2),whilst the network analysis were performed using the R packages EpiContactTrace (version 0.8.8) [40, 41] and iGraph (version 0.7.1) [42]. To calculate the skewness of the metric distributions, the package e1701 (version 1.6-4) [43] was used.

Ethics

The presented study was based on historical data from the AMD and the federal veterinary service. The data was anonymized for the analysis and legal requirements for the protection of data privacy were respected. No live animals were involved in the study. Therefore the study did not require the approval of an ethics committee.

Results

The seasonal fluctuation in the cattle trade network is reflected in the number of active nodes, the composition of premise types and the number of movements in the monthly networks. The months of February and July had the fewest movements and active premises. June and September had the highest number of movements and active premises. The number of nodes and movements in the networks considered are given in table 2.

About 75% of the cattle born before January 1st 2012 stayed in the same herd, while one fourth had been moved by the end of the year. Over the summer months, the proportion of animals leaving the herd increases because entire herds are moved to summer pasture. The increase in October (figure 2) is due to cattle returning from summer pasture.

Network metrics in the yearly and monthly networks

The distributions for the metrics studied are presented in detail for farms only, as those are the premises of interest for risk-based surveillance. The distributions in question are heavily skewed. Most farms have very few direct contacts and a few farms have many. The distribution of the ICC and OCC in the yearly network is negatively skewed, indicating that in a longer observation window, most holdings are connected to many other holdings (table 3).

The reachability ratios indicate, that in the shorter observation window of a month, only few farms are reachable (median in-reR = 0). Outgoing contacts are more frequent but lead to shorter chains than the ingoing contacts. If the temporal paths are observed over a year, the network gets more connected and the max reR's reach values above 80% (table 4).

The other holding types have different distributions by nature of their role in the network. The maximum value for each metric and holding type in the monthly network allows the comparison of the different activities (figure 3, see discussion).

Farm level parameters

The ICC_{max}, the ID_y, the avAN and the NS have highly right-skewed distributions (g_1 of 3.8, 13.26, 5.9, 4.75 and respectively) (figure 4).

The majority of farms move cattle every second week or less; 50% had registered movements in less than 16 weeks (figure 4). Half of the farms (49.9%) placed cattle on shared alpine pastures in 2012.

The number of active weeks had a stronger correlation to those criteria than the herd size. The average number of cattle per movement (avAN) had very weak correlations to the other selected criteria. The herd size has a correlation above 0.5 only with the AW. Given the strong correlation of the ICC_{max} and the NS (rho > 0.75), we decided to keep the ICC_{max} for the final scoring of the farms (table 5, see discussion).

The number of farms is presented in table 6 according to their score at the different thresholds.

Validation

When applying the score system to the farms with known BVD status in 2013, some substantial differences were observed. With the 50pct threshold, no farms with a new infection have a score 0 or 1) and only 10% of these farms have a score of 2. With the higher threshold levels, few farms of either status have scores of 3 or higher. However, at the most 20% of the positive farms have a score of 0 (figure 5).

Taking a score of ≥2 at the threshold level of 50% as criteria for sampling results in 100% sensitivity (assuming perfect test sensitivity at herd-level). The specificity at the same values is 36.54% (table 7).

Discussion

Our results show that farm level parameters based on animal movements can support risk-based selection of farms for surveillance programmes in Switzerland. The actual threshold needs to be chosen in function of surveillance goals, available budget and available data for validation. In the case of BVD surveillance, target farms with a score count of ≥ 2 at the lowest threshold levels would provide the highest sensitivity and all positive herds would be included in the sample. In the final stage of the BVD eradication programme, it is crucial to find the remaining domestic cases and therefore a high sensitivity and coverage is more important than the resulting number of negative farms tested. Also, reconfirming the free status increases the overall security of success of the eradication programme. However, it also means that the farms below the decision point of a score of 2 can be excluded from the sample. If the risk score had been applied to all active farms in 2012, this would translate in 10'400 farms (1/4 if the population) with a score count of 0 or 1 (table 6). For these farms, surveillance could be reduced to passive, or active sampling could be conducted with longer time intervals. The farms used for the validation have a well-known BVD status. If the objective of the scoring system is to detect farms with newly acquired PI animals, then the added uncertainty of an imperfect testing system must be taken into account.

The Swiss cattle industry operates in a small but densely populated area. Distances are short with less than 4 hours' drive from one end of the country to the other, although certain valleys where livestock are kept are relatively remote. Therefore, most traders (category 'market' in the study) operate on a national level. This leads to high levels of ingoing and outgoing contact chain values for most of the farms in the

network over time, although only very few farms (ca. 10 %) have high levels of direct contacts. This is reflected in the reR's, stating that in median, over 60% of all holdings are in the in- and output domain of any farm in the yearly network. The fact that the ingoing contact chains are generally bigger than the outgoing contact chains can possibly be explained by the different purposes of buying or selling animals: animals sold are mostly intended for slaughter (with possible few stops on the way at a fattening plant or cattle traders). Traders for slaughter animals are mostly specialized and buy directly from the farms. In contrast, the purchased animals are for restocking purposes, and probably more often acquired at fairs or from major cattle traders who have a big network of potential sellers and buyers.

The seasonal variation in the network parameters is driven by the pasturing season. 2012 was the first year for which reliable data on the movements from and to alpine pastures was available for Switzerland and to our knowledge the present study is the first to analyse these movements in detail.

The strong seasonal pattern suggests highly variable transmission risks during the year. It also illustrates that the time of sampling must be considered according to the goal of the surveillance programme.

To assess the importance of the position of a farm within the movement network, two temporal measures were used; the number of weeks with registered movements (AW) to find the farms with above average activity over time, and the fraction of shortest temporal paths a farm was on. A temporal analogy to the betweenness was also proposed by Kim and Anderson [44]. For a real live cattle network, an iterative approach is not necessary if appropriate time windows are used. To avoid confusion we used the abbreviation NS for the fraction of times a farm was on the shortest path instead of the term 'temporal betweenness'.

The methodological relationship of the ICC and NS is quite obvious, as the ICC also traces shortest paths through the temporal network. A farm with a large ICC and at least one outgoing contact is inevitably also on many of the shortest paths. But the ICC represents the farm as end point and the NS counts how many times it can be the connection between two other holdings. This gives the two measures different meanings, but they are nonetheless highly correlated. The NS was calculated for the first time for this study and its value for risk-based surveillance is not yet investigated with disease data. For the ICC the value for risk based surveillance was shown by Frössling et al. [7]. For future applications both measures may have their value depending on the underlying problem.

The weak correlation between herd size and the movement related parameters implies that they do not substitute one another as risk factors. Whether the herd size is added as criteria to the scoring system must be decided depending on the disease in question.

Finding measures to describe the position of a farm in temporal networks is challenging. The farms are only active in the trade network on a few days during the year and the possible contact patterns are countless. We believe that with the combination of the proposed criteria, we introduced a system that covers several features of the movement patterns for ranking the farms in a yearly time window. If applied on a yearly basis, the information gained on every farm will also improve the system. A further application of the score could be to better describe the risk of farms to get infected through animal movements in the risk-based surveillance for IBR and EBL, and to combine this score with the other risk factors.

Other studies have shown that network parameters are useful for risk-based surveillance. Frössling et al. showed that high ID and ICC are risk factors for the

occurrence of bovine corona virus but not for bovine respiratory syncytial virus [7]. In a recent study, the same group introduced a method for calculating the probability of disease ratio (PDR), a disease specific relative ratio of the increased probability of infection due to the introduction of animals [45]. Ribeiro-Lima et al. identified farms with a higher risk for bTB infection using a model based on a risk score at movement level [46]. These studies show the importance of validating proposed risk-scores for every disease in question.

For the study to be relevant for the Swiss veterinary authorities at present, the proposed framework must be applicable for BVD, BT, IBR or EBL. As Switzerland is free of IBR, EBL and BT, a validation for these diseases was not possible. BT would in any case be an unfit example for the validation as it is not a disease limited to cattle and its spread is attributed to vector activity, transport of infected vectors as well as animal movements [47, 48]. Additionally, the transmission dynamics of BVD between herds is relatively well known. The investigations after positive test results in the later stage of the eradication programme showed that BVD was introduced by cattle movements at least in some cases in Switzerland [49]. The risk of BVD infection of pregnant heifers on summer pasture, resulting in the birth of PIs on the home farm, is well established [49–54].

Only 29 farms had a PI animal following a new infection in 2013. The observed difference of scores of positive farms is therefore more influenced by the results of a single farm than for negative farms. As we are looking for a framework which is robust enough to select farms with a higher risk in absence of known disease cases, the presented results are encouraging. Noticeably, none of the farms with a PI has a score below three when using the lower threshold.

With the introduced scoring system, the information contained in the AMD can be used to optimize the selection of farms in the sample for routine surveillance. However, more data is needed to quantify the risk associated with the chosen criteria for other diseases such as IBR and EBL).

It can nonetheless help to choose farms for surveillance with a semi-quantitative framework using the available information and including experiences from other countries.

While this study aims at providing a framework for planning yearly surveillance programmes, other applications are possible. The most important might be to select farms for screening of cattle for contagious pathogens at slaughter. With the introduction of an information system to sample pre-selected cattle at the slaughterhouse, a surveillance component that allows continuous monitoring at relatively low costs would be available. By screening cattle at slaughter from farms with high scores, the framework could be implemented for monitoring programmes or at least provide the necessary data to validate the system itself. The high values of ID and ICC of slaughterhouses (see figure 3 for maximum values) throughout the year give confidence in the representativeness of samples taken at slaughter.

Conclusions

With the suggested framework, the information within the AMD can be used to optimize the selection of farms for risk-based surveillance. It is valid for the selection of farms with a higher risk of infection with bovine viral diarrhoea (BVD) due to their position in the trade network, but more data (or if not available, models) are needed to validate the approach for other diseases.

The seasonality and time dependency of the activity of single farms in the networks requires a careful assessment of the time period included to determine farm level parameters.

Abbreviations

AMD: animal movement database; avAN: Average number of animals per incoming movement of the farm in consideration; AW: active weeks; BT: bluetongue; BVD: bovine viral diarrhoea; EBL: enzootic bovine leucosis; IBR: infectious bovine rhinotracheitis; ICC: ingoing contact chain; ID: in-degree; MA: mixed alpine pasture; NS: number of shortest path (sequence of movements is considered) in which the node is present; OCC: outgoing contact chain; OD: out-degree; reR: reachability ratio

Competing interest

The authors declare that they have no competing interests.

Author's contribution

MR, HS, AL, JZ and SS participated in the design of the study. SW and SS performed the network analysis. SS, HS and MR built and validated the scoring system. MR, HS, BV and SS drafted the manuscript and all authors contributed to and approved the final version.

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Refrences

1. Wentholt M, Cardoen S, Imberechts H, Van Huffel X, Ooms B, Frewer L: **Defining European preparedness and research needs regarding emerging infectious animal disease: results from a Delphi expert consulation**. *Prev Vet Med* 2012, **103**:81–92.

2. Firestone S, Ward M, Christley R, Dhand N: **The importance of location in contact networks: Describing early epidemic spread using spatial social network analysis**. *Prev Vet Med* 2011, **102**:185–195.

3. Green DM, Kiss IZ, Mitchell AP, Kao RR: **Estimates for local and movement-based transmission of bovine tuberculosis in British cattle.** *Proc Biol Sci* 2008, **275**:1001–1005.

4. Cowiea C, Marreosb N, Gortázarb C, Jarosob R, Whitea P, Balseiroc A: **Shared risk factors for multiple livestock diseases: A case study of bovine tuberculosis and brucellosis**. *Res Vet Sci* 2014, **97**:491:497.

5. Junling M, van den Driessche P, Willeboordse F: **The importance of contact network topology for the success of vaccination strategies.** *J Theor Biol* 2013, **325**:12–21.

6. Ortiz-Pealez A, Guitian F, Pfeiffer D, Soares-Magalhães R, FJ G: **Use of social network analysis to characterize the pattern of animal movements in the initial phases of the 2001 foot and mouth disease (FMD) epidemic in the UK**. *Prev Vet Med* 2006, **76**:40–55.

7. Frössling J, Ohlson A, Björkman C, Håkansson N, Nöremark M: **Application of network analysis parameters in risk-based surveillance - examples based on cattle trade data and bovine infections in Sweden.** *Prev Vet Med* 2012, **105**:202–208.

8. Martinez-López B, Perez A, Sánchez-Vizcaíno J: **Social Network Analysis. Review of Genera Concepts and Use in Preventive Veterinary Medicine.** *Transbound Emerg Dis* 2009, **56**:109–120.

9. Dubé C, Ribble C, Kelton D, Mcnab B: **A Review of Network Analysis Terminology and its Application to Foot-and-Mouth Disease Modelling and Policy Development**. *Transbound Emerg Dis* 2009, **56**:73–85.

10. Dubé C, Ribble C, Kelton D, Mcnab B: **Introduction to network analysis and its implications for animal disease modelling**. *Rev Sci Tech* 2011, **30**:425–436.

11. Luke A, Harris J: **Network analysis in public health: History, Methods and applications**. *Annu Rev Public Health* 2008, **28**:69–93.

12. Natale F, Giovannini A, Savini L, Palma D, Possenti L, Fiore G, Calistri P: **Network analysis of Italian cattle trade patterns and evaluation of risks for potential disease spread**. *Prev Vet Med* 2009, **92**:341–350.

13. Grisi-Filho J, Amaku M, Ferreira F, Dias R, Neto J, Negreiros R, Ossada R: **Detecting livestock production zones**. *Prev Vet Med* 2013, **110**:304–3011.

14. Nöremark M, Håkansson N, Sternberg S, Lindberg A, Jonsson A: **Network analysis of cattle and pig movements in Sweden : Measures relevant for disease control and risk based surveillance**. *Prev Vet Med* 2011, **99**:78–90.

15. Holme P, Saramäki J: **Temporal networks**. *Phys Rep* 2012:97–125.

16. Flocchini P, Mans B, Santoro N: **On the exploration of time-varying networks**. *Theor Comput Sci* 2013, **469**:53–68.

17. Blonder B, Wey TW, Dornhaus A, James R, Sih A: **Temporal dynamics and network analysis**. *Methods Ecol Evol* 2012:958–972.

18. Riolo CS, Koopman JS, Chick SE: **Methods and measures for the description of epidemiologic contact networks.** *J urban Heal Bull New York Acad Med* 2001, **78**:446–457.

19. Reist M, Jemmi T, Stärk K: **Policy-driven development of cost-effective, risk-based surveillance strategies.** *Prev Vet Med* 2012, **105**:176–84.

20. Hadorn D, Rüfenacht J, Hauser R, Stärk K: **Risk-based design of repeated surveys for the documentation of freedom from non-highly contagious diseases**. *Prev Vet Med* 2002, **56**:179–192.

21. Schwermer H, Reding I, Hadorn D: **Risk-based sample size calculation for consecutive surveys to document freedom from animal diseases**. *Prev Vet Med* 2009, **92**:366–372.

22. Stärk K, Regula G, Hernandez J, Knopf L, Fuchs K, Morris R, Davies P: **Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health : Review of current approaches**. *BMC Health Serv Res* 2006, **6:20**.

23. Blickenstorfer S, Schwermer H, Engels M, Reist M, Doherr MG, Hadorn DC: **Using scenario tree modelling for targeted herd sampling to substantiate freedom from disease.** *BMC Vet Res* 2011, **7**:49.

24. Gates M, Humphry RW, Gunn GJ, Woolhouse MEJ: **Not all cows are epidemiologically equal: quantifying the risks of bovine viral diarrhoea virus (BVDV) transmission through cattle movements**. *Vet Res* 2014, **45**:110.

25. Brock K V.: **The persistence of bovine viral diarrhea virus**. In *Biologicals*. *Volume 31*; 2003:133–135.

26. Schwermer H, Bernet D, Presi P, Schaller P, Stern M, Heim D: **Data management systems for the bovine viral diarrhoea eradication programme in Switzerland.** *Rev Sci Tech* 2013, **32**:741:745.

27. Reber A, Reist M, Schwermer H: **Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhino- tracheitis and bovine enzootic leucosis in Switzerland**. *SAT* 2012, **154**:189–197.

28. Ståhl K, Kampa J, Baule C, Isaksson M, Moreno-López J, Belák S, Alenius S, Lindberg A: **Molecular epidemiology of bovine viral diarrhoea during the final phase of the Swedish BVD-eradication programme**. In *Prev Vet Med*. *Volume 72*; 2005:103–108.

29. Lindberg A, Groenendaal H, Alenius S, Emanuelson U: **Validation of a test for dams carrying foetuses persistently infected with bovine viral-diarrhoea virus based on determination of antibody levels in late pregnancy**. *Prev Vet Med* 2001, **51**:199–214.

30. Tinsley M, Lewis FI, Brülisauer F: **Network modeling of BVD transmission**. *Vet Res* 2012, **43**:11.

31. Voelk V, Graber HU, van den Borne BHP, Sartori C, Steiner A, Bodmer M, Haerdi-Landerer MC: **A longitudinal study investigating the prevalence of Staphylococcus aureus genotype B in seasonally communal dairy herds.** *J Dairy Sci* 2014, **97**:4184–4192.

32. **agate** [https://www.agate.ch/portal/web/agate/home]

33. Wasserman S, Faust K: *Social Network Analysis: Methods and Applications (Structural Analysis in the Social Sciences)*. *Volume 4*. New York: Cambridge University Press; 1994:857.

34. Webb CR: **Investigating the potential spread of infectious diseases of sheep via agricultural shows in Great Britain.** *Epidemiol Infect* 2006, **134**:31–40.

35. Dutta BL, Ezanno P, Vergu E: **Characteristics of the spatio-temporal network of cattle movements in France over a 5-year period**. *Prev Vet Med* 2014, **117**:79–94.

36. Joanes DN, Gill CA: **Comparing measures of sample skewness and kurtosis**. *J R Stat Soc Ser D (The Stat* 1998, **47**:183–189.

37. Cameron A: **The consequences of risk-based surveillance: Developing output-based standards for surveillance to demonstrate freedom from disease.** *Prev Vet Med* 2012, **105**:280–286.

38. Freeman LC: **Centrality in social networks conceptual clarification**. *Soc Networks* 1978:215–239.

39. Dohoo I, Wayne M, Stryhn H: *Veterinary Epidemiologic Research*. 2nd edition. VER Inc.; 2010.

40. Nöremark M, Widgren S: **EpiContactTrace: an R-package for contact tracing during livestock disease outbreaks and for risk-based surveillance.** *BMC Vet Res* 2014, **10:71**.

41. R Development Core Team: **R: A language and environment for statistical computing.** 2008.

42. Csardi G, Nepusz T: **The igraph software package for complex network research**. *InterJournal* 2006, **Complex Sy**:1695.

43. Meyer D, Dimitriadou E, Hornik K, Weingessel A, Leisch F: **e1071: Misc Functions of the Department of Statistics (e1071). R package version 1.6-4.** 2014.

44. Kim H, Anderson R: **Temporal node centrality in complex networks**. *Phys Rev E - Stat Nonlinear, Soft Matter Phys* 2012, **85**(November 2011):1–8.

45. Frössling J, Nusinovici S, Nöremark M, Widgren S, Lindberg A: **A novel method to identify herds with an increased probability of disease introduction due to animal trade**. *Prev Vet Med* 2014, **117**:367–374.

46. Ribeiro-Lima J, Enns E a., Thompson B, Craft ME, Wells SJ: **From network analysis to risk analysis—An approach to risk-based surveillance for bovine tuberculosis in Minnesota, US**. *Prev Vet Med* 2015, **118**:328–340.

47. Ensoy C, Aerts M, Welby S, Van Der Stede Y, Faes C: **A dynamic spatio-temporal model to investigate the effect of cattle movements on the spread of bluetongue BTV-8 in Belgium**. *PLoS One* 2013, **8**.

48. De Koeijer AA, Boender G, Nodelijk G, Staubach C, Meroc E, Elbers ARW: **Quantitative analysis of transmission parameters for bluetongue virus serotype 8 in Western Europe in 2006**. *Vet Res* 2011, **42**.

49. Presi P, Struchen R, Knight-Jones T, Scholl S, Heim D: **Bovine viral diarrhea (BVD) eradication in Switzerland-Experiences of the first two years**. *Prev Vet Med* 2011, **99**:112–121.

50. Siegwart N, Hilbe M, Hässig M, Braun U: **Increased risk of BVDV infection of calves from pregnant dams on communal Alpine pastures in Switzerland**. *Vet J* 2006, **172**:386–388.

51. Bodmer M, Kaufmann T, Zanoni R, Steiner A: **A BVD free communal alpine pasture in Switzerland**. In *Cattle Pract*. *Volume 15*; 2007:294.

52. Rossmanith W, Janacek R, Wilhelm E: **Control of BVDV-infection on common grassland - The key for successful BVDV-eradication in Lower Austria**. In *Prev Vet Med*. *Volume 72*; 2005:133–137.

53. Braun U, Schönmann M, Ehrensperger F, Hilbe M, Strasser M: **Intrauterine infection with bovine virus diarrhoea virus on alpine communal pastures in Switzerland.** *Zentralbl Veterinarmed A* 1999, **46**:13–17.

54. Braun U, Schönmann M, Ehrensperger F, Hilbe M, Brunner D, Stärk KD, Giger T: **Epidemiology of bovine virus diarrhoea in cattle on communal alpine pastures in Switzerland.** *Zentralbl Veterinarmed A* 1998, **45**:445–452.

Tables

Table 1: Farm level criteria linked to cattle movement with importance for disease surveillance

Name	Description
ID_y	ID over the entire year
avAN	Average number of animals per incoming movement of the farm in consideration
ICC_{max}	Maximum ICC over the twelve monthly networks
NS	Fraction of times a premise is on the shortest temporal path between two premises of all existing shortest temporal paths in the given time window
MA	Sent animals to alpine pastures with more than one farm of origin (yes/no)
AW	Number of weeks with registered movements

Table 2: Yearly and monthly networks in 2012. The numbers of active holdings (nodes) are recorded as total and per holding type. The number of movements (arcs) is given as total. SH: slaughterhouse.

Network	Number of active holdings						Number of Movements
	Total	Alp	Clinic	Farm	Market	SH	
Yearly	48’728	6’451	5	41’484	189	599	907’539
January	30’525	35	4	29’831	112	543	70’160
February	29’674	34	3	28’997	112	528	59’860
March	30’749	32	3	30’069	112	533	71’278
April	31’047	109	4	30’280	118	536	68’306
May	35’806	2’841	5	32’311	111	538	79’869
June	39’512	5’506	5	33’369	111	521	90’880
July	29’556	3’399	4	25’573	99	481	59’925
August	32’401	4’174	3	27’629	105	490	72’844
September	40’300	5’829	3	33’851	126	491	103’587
October	36’190	2’763	2	32’782	121	522	89’693
November	32’978	248	2	32’092	120	516	78’612
December	28’770	27	4	28’130	111	498	62’525

Table 3: Yearly and monthly network metrics, ID: In-degree; OD: out-degree; ICC: ingoing contact chain; OCC: outgoing contact chain for in 201. Mean, median, g_1: skewness. Max: maximum are provided.

network	ID				OD				ICC				OCC			
	mean	median	max	g_1	mean	median	max	g_1	mean	median	max	g_1	mean	median	max	g_1
Yearly	7.75	3	893	13.26	10.44	8	302	6.27	28’051.85	30’673	45’740	-0.91	29’808.64	3’8247	40’848	-1.16
January	1.12	0	211	15.39	2.01	2	103	10.55	73.25	0	3’525	4.87	83.90	3	2’165	4.98
February	1.03	0	250	21.27	1.94	1	42	5.81	28.62	0	12’822	35.58	35.26	5	1’368	5.25
March	1.12	0	231	16.72	2.01	2	49	5.92	44.89	0	7’632	7.49	53.11	3	1’858	5.78
April	1.13	0	199	16.80	1.97	2	51	6.18	67.11	0	4’017	7.27	76.79	3	1’832	3.92
May	1.07	0	278	22.77	2.13	2	49	5.50	30.43	0	5’473	8.59	41.69	3	2’478	9.04
June	0.92	0	227	20.11	2.35	2	58	5.39	29.38	0	4’745	9.21	41.23	4	2’279	5.63
July	1.04	0	209	18.76	1.80	1	83	10.09	14.55	0	4’201	12.31	17.44	2	892	6.32
August	1.27	1	216	16.52	1.84	1	56	6.26	25.32	1	2’856	8.85	26.56	2	1’218	6.59
September	1.86	1	167	15.16	1.70	1	56	5.94	107.39	1	10’608	5.83	89.21	3	3’192	5.58
October	1.48	1	337	22.34	2.06	2	56	6.00	111.45	1	3’898	4.55	105.72	3	2’833	4.74
November	1.26	0	262	19.11	2.16	2	71	6.56	50.69	0	9’492	9.16	58.15	4	2’196	5.26
December	1.10	0	250	22.57	1.92	1	49	5.34	58.33	0	2’627	5.50	65.47	2	2’400	5.28

Table 4: Yearly and monthly network metrics: In-reR: ingoing reachability ratio; out-reR: outgoing reachability ratio for farms in 2012. Mean, median; b_1: skewness; max: maximum are provided.

network	in- reR				out-reR			
	mean	median	max	b1	mean	median	max	b1
Yearly	0.5757	0.6295	0.9387	-0.9053	0.6117	0.7849	0.8383	-1.1604
January	0.0024	0.0000	0.1155	4.8747	0.0027	0.0001	0.0709	4.9819
February	0.0010	0.0000	0.4321	35.5800	0.0012	0.0002	0.0461	5.2501
March	0.0015	0.0000	0.2482	7.4938	0.0017	0.0001	0.0604	5.7800
April	0.0022	0.0000	0.1294	7.2695	0.0025	0.0001	0.0590	3.9152
May	0.0008	0.0000	0.1529	8.5878	0.0012	0.0001	0.0692	9.0394
June	0.0007	0.0000	0.1201	9.2075	0.0010	0.0001	0.0577	5.6280
July	0.0005	0.0000	0.1421	12.3136	0.0006	0.0001	0.0302	6.3163
August	0.0008	0.0000	0.0881	8.8484	0.0008	0.0001	0.0376	6.5948
September	0.0027	0.0000	0.2632	5.8321	0.0022	0.0001	0.0792	5.5803
October	0.0031	0.0000	0.1077	4.5503	0.0029	0.0001	0.0783	4.7412
November	0.0015	0.0000	0.2878	9.1592	0.0018	0.0001	0.0666	5.2620
December	0.0020	0.0000	0.0913	5.5001	0.0023	0.0001	0.0834	5.2790

Table 5: Correlation matrix for the considered farm level criteria (using Spearman rank correlation coefficients)

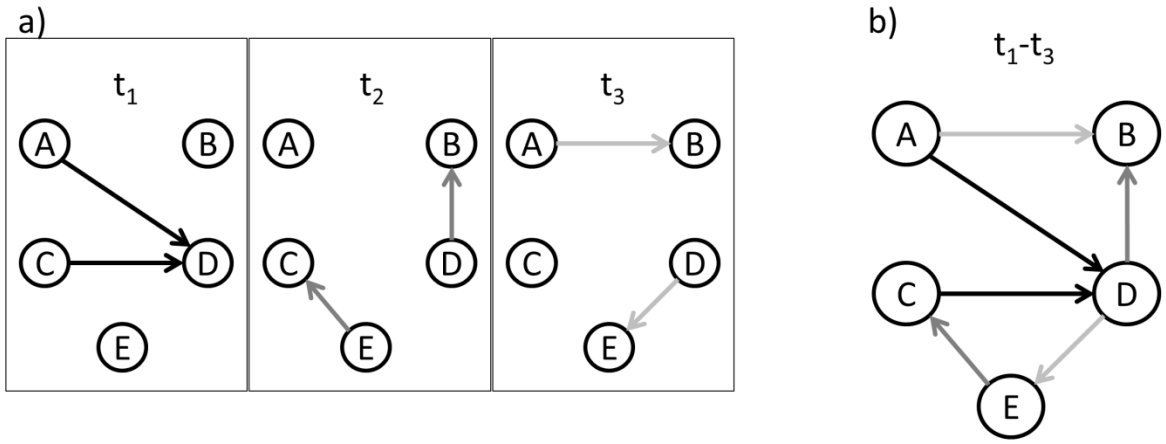
	ICC_{max}	ID_Y	NS	avAN	AW	Herd size
ICC_{max}	1	0.71	0.82	0.1	0.53	0.29
ID_Y	0.71	1	0.67	0.16	0.64	0.31
NS	0.82	0.67	1	0.13	0.64	0.39
avAN	0.1	0.16	0.13	1	0.14	0.18
AW	0.53	0.64	0.64	0.14	1	0.69
Herd size	0.29	0.31	0.39	0.18	0.69	1

Table 6: Number of farms according to their score and the four threshold values (50%, 90%, 95% and 99% quantile) considered.

	Threshold			
score	50%	90%	95%	99%
0	5'192	15'880	17'745	19'298
1	5'208	16'378	19'051	20'485
2	8'039	6'865	3'848	1'535
3	9'731	1'759	673	134
4	7'342	594	166	31
5	5'971	7	0	0

Table 7: Sensitivity (Se) and specificity (Sp) of detecting the farms with new infections with the subset of truly negative and known positive farms for different score counts and thresholds of 50% and 95%. FP: false positive; TP: true positive; FN: false negative; TN: true negative.

	Threshold									
	50%					95%				
Score count	≥ 1	≥2	≥3	≥4	≥5	≥1	≥2	≥3	≥4	≥5
FP	.	962	604	312	111	778	114	4	1	0
TP	.	29	26	20	11	23	11	3	1	0
FN	.	0	3	9	18	6	18	26	28	29
TN	.	554	912	1204	1405	738	1402	1512	1515	1516
Se	.	100.00%	89.66%	68.97%	37.93%	79.31%	37.93%	10.34%	3.45%	0.00%
Sp	.	36.54%	60.16%	79.42%	92.68%	48.68%	92.48%	99.74%	99.93%	100.00%



c)

Node	ID	OD	ICC	OCC
A	0	2 (B, D)	0	3 (B, D, E)
B	2 (A, D)	0	3 (A, C, D)	0
C	1 (E)	1 (D)	1 (E)	3 (B, D, E)
D	2 (A, C)	2 (B, E)	2 (A, C)	2 (B, E)
E	1 (D)	1 (C)	3 (A, C, E)	1 (C)

Figure 1: Illustration of a temporal network.

a) Three time steps (t_1, t_2, t_3) in a schematic temporal network. In every time step, two movements between holdings take place. b) the same network over the time period t_1- t_3. The network metrics ID, OD, ICC and OCC are calculated for every node in this network. c) Table with the network metrics for every node in the temporal network. Note that paths can only be built from darker to lighter colours of the arcs.

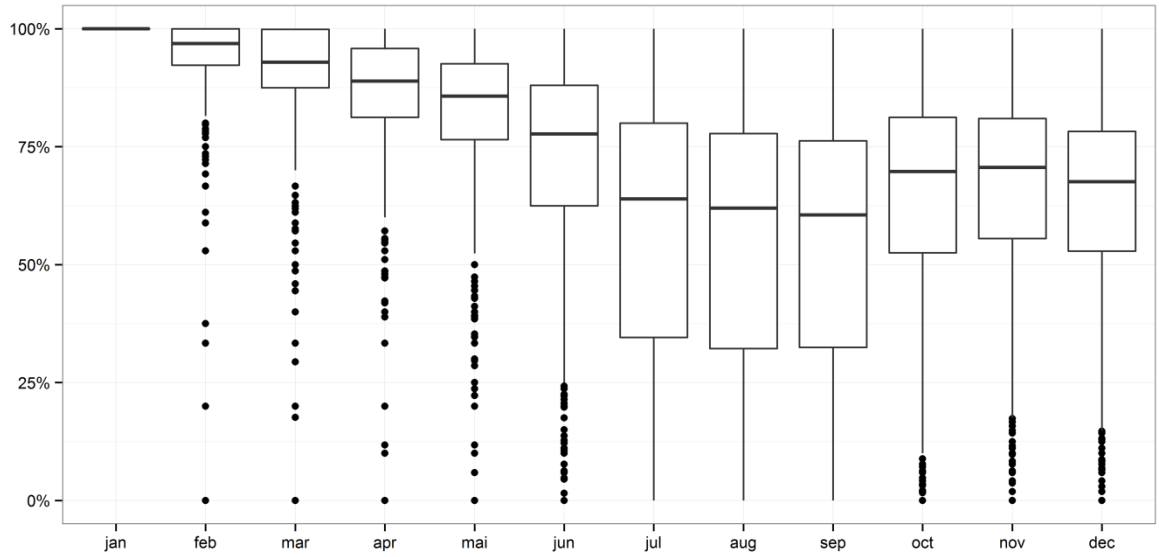


Figure 2: The January cohort followed over one year.

The proportion expresses how many cattle were still in the same herd on the 1st of every month in 2012. Over the summer month, the proportion of animals leaving the herd increases because entire herds are moved to summer pasture. The increase of bovines originally in the herd in October is due to cattle returning from summer pasture.

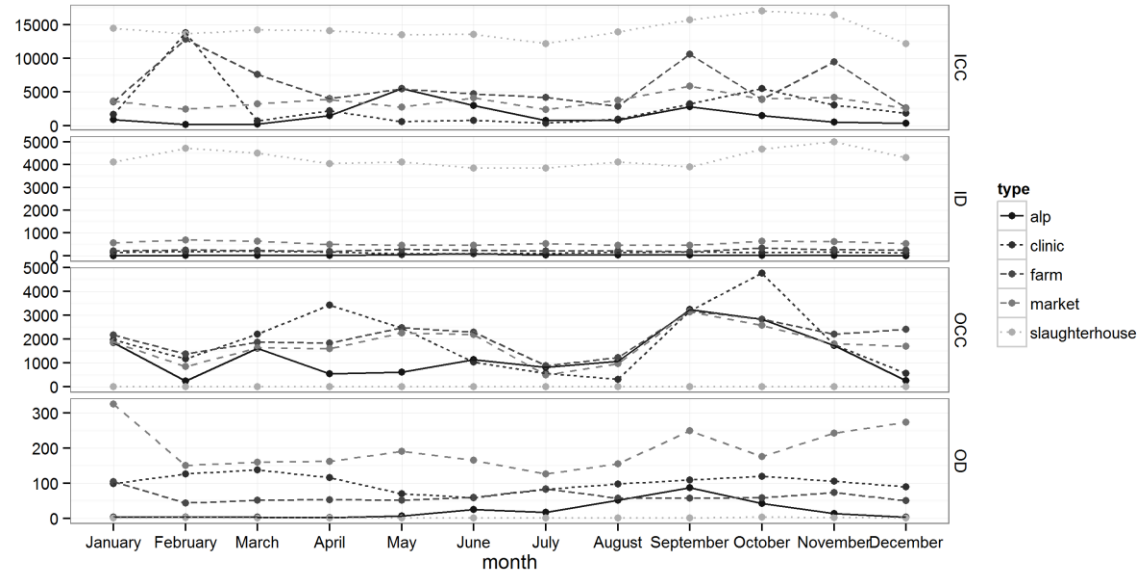


Figure 3: Maximum ID, OD, ICC and OCC for the different holding types in the Swiss cattle trade network in 2012.

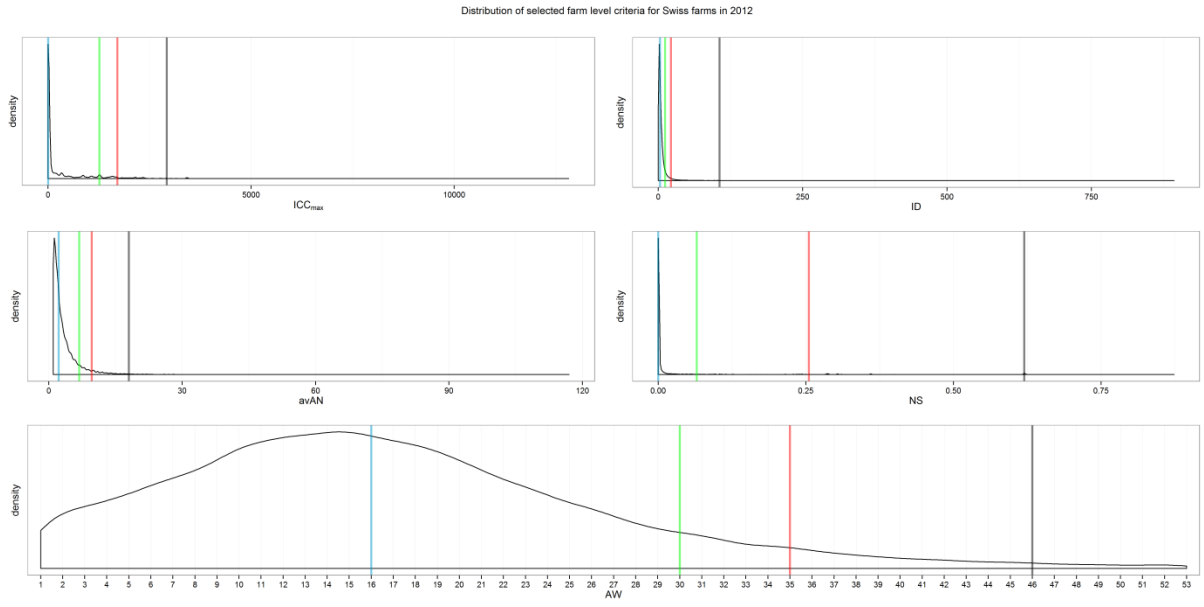


Figure 4: Probability density functions of the farm level criteria considered (ID_y, ICC_{max}, NS, avAN, AW).

Data from 2012 in Switzerland is presented. The applied thresholds are shown as vertical lines: skyblue: 50% quantile; green: 90% quantile; red: 95% quantile; grey: 99% quantile;

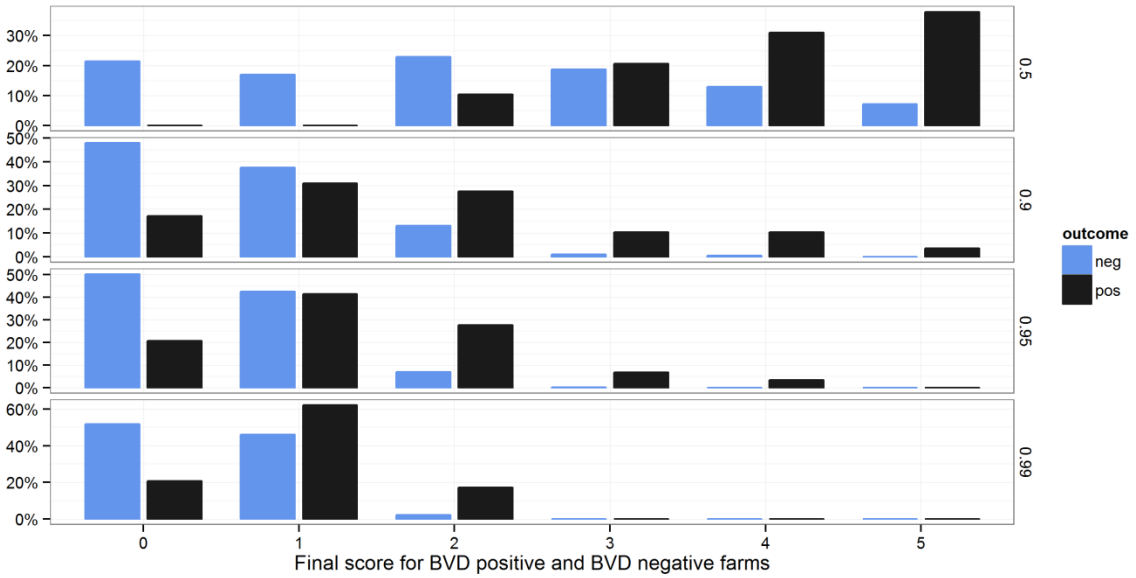


Figure 5: Proportion of farms with the same score count for different thresholds.

Blue: farms that never had a suspicious BVD result since the beginning of the eradication programme; black: farms in the BVD surveillance programme 2013 and at least one PI.

8 Feasibility of slaughterhouse sampling for surveillance of beef cattle in Switzerland

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Abstract

On grounds of potential economic benefits, the feasibility of transferring the sampling of the cattle population from on-farm to the slaughterhouse is assessed. The present study evaluates the possibilities of (i) sampling different matrices at different positions of the slaughter chain, (ii) sampling animals according to given criteria, and (iii) identifying predetermined animals for sampling. In collaboration with the six Swiss cattle slaughterhouses with the highest slaughter volume, on-site inspections, a workshop with stakeholders, a pilot study and the retrospective evaluation of the bluetongue surveillance programme 2011 were conducted. The results show, that relocation of the sampling of the Swiss cattle population to the slaughterhouses is possible. However, due to a lacking centralized data management system enabling real–time data exchange and missing technical aids for the identification of preselected animals, the possibilities for risk-based or even herd-level surveillance are very limited.

Code words: epizootics, slaughterhouse, surveillance, cattle, feasibility, sampling

Introduction

In Switzerland, the national regulation (Anonymous, 1995), the bilateral treaties with the EU (Anonymous, 1999) and the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) specify rules for food safety and animal and plant health standards. Early detection of disease, monitoring of present agents and verification of freedom from disease are described as key tasks of modern public veterinary services in order to allow international trade with animals and agricultural goods and to document the sanitary status of domestic livestock.

The necessity of costly surveillance systems for the described purposes contrasts with limited financial and personal resources (Reist et al., 2012). To optimize the costs, while maintaining the internationally agreed standards, different strategies are adopted. The sample size and therefore analytic cost can be reduced by applying risk-based approaches (Stärk et al. 2006, Reist et al. 2012). To reduce manpower and to optimize logistics for the sampling itself, the use of centralized infrastructures where enough representative specimens for large scale sampling are available seems obvious. In Switzerland combinations of both approaches are used. While the Swiss pig and poultry population are monitored in the slaughterhouse at least partly using risk-based approaches for selecting herds (Corbellini et al. 2006, Gohm et al. 1999), dairy cattle is tested using bulk milk samples provided by the laboratories conducting the biweekly mandatory milk quality control (Hadorn et al. 2009, Reber, 2012). Although bulk milk samples are a cost-saving alternative to on-farm blood sampling, there are intrinsic limitations as only farms that deliver commercial milk and hence only lactating cows are covered. Furthermore, not all relevant diagnostic tests for antibody or antigen detection can be performed on milk. To overcome these drawbacks, the remaining beef population or - if necessary - segments from dairy farms are covered by individual blood testing on farms. Individual blood sampling is always laborious, particularly the sampling of beef cattle. The husbandry of cattle for meat production involves far less human interaction compared to dairy cows and mother cows tend to defend their calves vigorously. It is also common to keep beef herds in semi-wild conditions, sometimes in considerable distances to the farms and on high altitude. This implies long travel time and a difficult and at times dangerous sampling procedure for the assigned veterinarians as the animals need to be

captured on the pastures. Assuming further shortening of resources, it is apparent that cost-efficient sampling procedures must be explored.

In contrast to slaughterhouses for other species than cattle, the cattle slaughterhouses in Switzerland are not yet fully exploited for monitoring and surveillance, which indicates that the present problems are nontrivial and need to be solved. Cattle are naturally clustered in herds (where animals from one farm are considered as a herd) and typically a two stage sampling procedure is applied as most of the mandatory programmes require conclusions on herd level (e.g. substantiation of freedom from infectious bovine rhinotracheitis [IBR] with 99% confidence at the 0.2% herd prevalence level). First, the number of animals per herd to be sampled to reach the targeted herd sensitivity is calculated, followed by the number of farms required to reach the goal of the survey. However, cattle are typically not slaughtered in batches and often insufficient numbers of animals from one herd are slaughtered on a single slaughtering day to achieve reasonable herd sensitivities to draw conclusions on herd level. To enable conclusions on herd level, animals of the same herd have to be relatable to each other, even if they arrive on different days and at different slaughterhouses. This is even more relevant for risk-based surveys where animals from targeted farms at risk or even pre-determined animals meeting certain risk factors need to be sampled.

Furthermore, the sample collection by the slaughterhouse staff must not disturb the slaughter process or bring additional accident hazard for the personnel. These practical challenges combined with the necessity to sample targeted animals meeting specific criteria (for example production type, age or - in the most difficult case – predetermined individuals to be sampled) require hands-on solutions to exploit the potential reduction of resources using slaughterhouses for the sampling of cattle.

The objective of the present study was to assess the feasibility of sampling cattle at Swiss slaughterhouses considering different sampling matrices, the necessity to reliably recognise animals meeting certain criteria (e.g. age, production type) and the feasibility of timely recognising individual animals predetermined to be sampled at different work stations along the slaughter line.

Material and Methods

For this study, the six largest of over 600 registered slaughterhouses in Switzerland were selected. They cover approximately 70% of the bovine slaughter population, e.g. 450'121 of 647'748 bovines slaughtered in 2009 (Figure 1). Their approximate slaughter volume accounts for 300 – 600 animals per day each, while most of the others slaughter only a few animals per week.

Slaughterhouse visits and workshop with stakeholders

All study slaughterhouses were visited from April to July 2011. During the visits, the slaughter line was assessed regarding possible sampling matrices and advantages and constraints of sampling at every workstation (see Table 1). Furthermore, the practical possibilities of sampling, the time effort, change in operational procedures and the recognition and identification of individual animals were considered. The results of the visits were discussed with official veterinarians from the slaughterhouses, from the competent cantonal veterinary offices and from the federal veterinary office (FVO) during a workshop. To describe the slaughter habits of Swiss farmers, the slaughter data from 2009 from the Swiss animal movement database (AMD) was summarized.

Since it had proven difficult to estimate the expenditure of sampling in the slaughterhouses without specifying the sample volume, matrix and time frame, the emphasis of the workshop was put on refining the results regarding the expenses for five working sampling scenarios to be discussed by the participants. For each scenario, a required volume of five samples from a total of 1500 beef farms was assumed. The scenarios varied in their specification on how the animals were identified and selected in the slaughterhouses and if the selection of suitable samples was handled by the slaughterhouse personnel, a central unit like the veterinary service or the laboratory or a data management system (see Table 2).

Pilot study

To assess the feasibility of sampling animals exclusively from the beef production with the currently available tools and infrastructure, a pilot study was conducted in two of the study slaughterhouses by two veterinarians. For a period of two weeks, blood samples were taken of all animals corresponding with the inclusion criteria: Beef cattle older than 6 months and at least 3 animals from one farm to assure a minimal sensitivity at farm level.

At the end of each day, the samples were cross-referenced with an extract from the AMD using Microsoft Access, Hence, samples from animals that did not meet the given criteria were discarded. The final samples were sent to the national reference laboratory for IBR and analysed serologically for IBR antibodies to simulate realistic conditions and to additionally evaluate the laboratory perspective on sample quality, feasibility of processing and potential weaknesses of the conducted programme. The sub-project was focused on the practical feasibility and on the identification of possible factors influencing the targeted composition of the surveyed population.

Evaluation of the Swiss bluetongue surveillance programme 2011

In the past, bluetongue (BT) monitoring in cattle was based on serological testing in bulk milk and blood serum in Switzerland. After having detected the first BTV-8 cases and having started a mandatory vaccination program in 2008, testing for antibodies was no longer informative for adult cattle. Since then, further monitoring programmes have focused on antigen detection in blood samples of young bovine stock that were too young to be vaccinated but lived long enough during the main vector period (Reist et al., 2012). In 2011, blood sampling was displaced from the farm to the slaughterhouse, and the programme was mainly based on antibody detection. To avoid the detection of maternal antibodies (as only newly infected animals are of interest), the commissioned slaughterhouses were given the task to take samples only from animals that were between 6 and 18 months of age. To gain a maximum of information about the population, not more than 6 animals from the same farm should have been sampled. The goal of the surveillance program was the proof of freedom from infection with a design prevalence of 0.2% on animal level. The necessary sample size to reach a sensitivity of 99% was 2550 animals (FVO, 2012).

This programme was retrospectively evaluated by analysing slaughter data from the AMD and the data on the samples (provided by the FVO), comparing the program requirements with the actually taken samples to assess the compliance of the slaughterhouses with the sampling specifications regarding age category and number of animals per farm. Additionally, questionnaires were sent to the concerned slaughterhouses to collect information about encountered difficulties in the timely identification of the target population along the slaughter chain for sampling.

Data management and analysis

The extract from the AMD (2007-2011) was stored in PostgreSQL (The PostgreSQL Global Development Group, 2013) and SQuirrel SQL was used as client for queries and data manipulations. Graphs and sampling scenarios were built using the statistical software R (R Development Core Team, 2011). For the sampling scenarios, the slaughter data from 2009 was used.

Results

Slaughterhouse visits and workshop with stakeholders

The slaughterhouse veterinarians agreed that only veterinarians or meat inspectors are qualified to take samples. As reasons for this constraint they listed lacking epidemiological background and motivation of line workers in the slaughterhouse to provide useful samples, as they not necessarily see the importance of national surveillance of the cattle population. Moreover, most mandatory monitoring programmes require samples to be taken by official veterinarians or meat inspectors. Consequently, only work stations where a veterinarian is already present or where enough space for an additional person is available can be used for sampling. Generally the bleeding, the meat inspection and the route between the scales and the chill rooms were identified as the most suitable positions for taking samples.

All sampling materials considered could basically be gained at some point in the slaughter line in all study slaughterhouses, but not all are equally convenient (Table 1). For large scale and routine surveillance programmes, blood and meat juice are the favoured sampling materials. All study slaughterhouses are experienced with blood sampling and follow their individual protocols. Sampling is performed at the site of bleeding or from the pericardium at meat inspection. Also samples from the central

nervous systems and all red organs (spleen, liver, kidneys, lung and heart) can be gained relatively easy and consistently in all study slaughterhouses.

The other considered matrices were not convenient for large scale sampling but rather for specific “one time” studies. For most of them, individual solutions for every slaughterhouse must be found.

In general, sampling living animals (e.g. for fur and nose or conjunctiva swabs) is not reckoned practicable for several reasons, although a veterinarian is present for the ante-mortem inspection at the stables; while unloading and driving the animals, the stress levels are high and there is no time to check for individual animals or sampling criteria. Furthermore, when cattle are stabled, singling out individuals is challenging if not impossible. Additionally, it is dangerous to sample corralled animals since possibilities for fixation are few. Waiting times for different animal groups varied before they enter the slaughtering process. White organs (rumen, stomachs, intestines, lymph nodes and reproductive organs including uterus) are likewise not accessible for large scale sampling without considerable additional workload. As they run on a separate conveyor belt in parallel to the red organs and the carcass, they are only identified indirectly as they pass the post mortem control simultaneously. For calves, white organs of two individuals are often put together on the conveyor belt and cannot be distinguished anymore. To select individual animals for sampling, the bowel would have to be marked before the tracks of the white and red organs and the carcass separate. Faecal samples are preferably taken from the intestines after the evisceration, but in addition to the difficulties in the identification of single animals at this stage, there is also a considerable hygiene issue by exposing bowel content in the clean zone of the slaughterhouse.

For sampling schemes where specific selection criteria must be met (e.g. risk based sampling or the sampling of only beef cattle) suitable animals must be recognized and/or labelled during the slaughter process. All animals are delivered with accompanying documents containing information about the farm of origin and the animal´s individual identification number (AMD Nb), which is also displayed on the ear tags allowing tracking the whole animal history as specified in the AMD. Before they enter the slaughter process, the information from the accompanying document is read into the computer system. It would be possible to mark animals at this stage (for example with coloured spray) or enter additional electronic information for the sampling to make it available at positions in the slaughter line where computers are present (in all study slaughterhouses at least at stunning, meat inspection and weighing). However, at present, the information systems of the slaughterhouses are not linked directly to the AMD. Hence, all information relevant for the sampling scheme would have to be available on the accompanying document. Today, the production types, herd size, breed or similar are not listed and therefore not available as selection criteria.

The sampling scenarios discussed during the workshop with the stakeholders were based on the slaughter data from 2009. The number of samples taken varied, from over 22’000 to 7’500 (1500 farms * 5 animals) depending on the sampling strategies defined by the scenarios. The sampling lasted between 3 and 12 months (Table 2). As a result of the discussion, only scenarios with either a temporally concentrated amount of samples (Scenario I) or with only a few samples per day were identified as being realistic. In the first case, a person could be temporarily employed exclusively for taking samples. In the second case, the present veterinarians can take the samples in addition to their daily workload. The sample limit of 20 samples per day

and slaughterhouse was considered low. According to the present veterinarians and varying with slaughterhouse and human resources available, 60-100 samples per day could be feasible without additional personnel. For the scenarios without a (hypothetical) central data management system (scenarios I-III), many farms would be sampled more than once because of the slaughter habits of Swiss farmers (Figure 2b).

In spite of the scenarios, the expense of the slaughterhouses for routine sampling programmes could only be estimated roughly, but there was a general consent that even a scenario with a few samples per day over a long period would result in additional labour time that would have to be compensated.

Pilot study

In the pilot study, the production type and age of the animals were verified using the AMD at the end of the slaughtering day and samples from animals from the dairy sector or animals older than 6 months where discarded. The veterinarians that carried out the sampling reported difficulties to recognize all animals that meet the inclusion criteria for sampling at the operating slaughter line.

In one of the two slaughterhouses where the blood was taken upon incision of the ventricles of the heart during meat inspection information about organic or animal friendly production was noted on the carcass label. This facilitated the selection of animals from beef producers. However, cows from suckle cow husbandry were not distinguishable from dairy cows without the fur as an indicator for the breed. In the other slaughterhouse, the blood was collected at bleeding.

In both slaughterhouses, it was not possible during the sampling to control the farm of origin.

Overall, 18.5% (209 of 1130) of the samples had to be thrown away when compared with the AMD because the sampling criteria were not met (min 3 animals per farm, older than 6 months and beef production).

The dispatching of the samples to the assigned laboratory and their analysis was neither problematic for blood gained from incision of the heart nor for blood gained during bleeding. The laboratories recommended an electronically instead of handwritten labelling of samples (e.g. QR codes) to ensure the readability of the labels.

Evaluation of the BT surveillance programme 2011

Four of the six assigned slaughterhouses returned the questionnaire. At one slaughterhouse, the blood samples were taken from the pericardium during the meat inspection, at all others at bleeding. The only reasons for discarding samples was noncompliance with the given selection criteria. The other possible reasons for discard of tubes, ”containing insufficient quantities of blood” or ”sampled animals not traceable in the AMD” did not cause any discard of tubes in this survey.

The selection of animals according to given criteria was considered difficult. In the participating slaughterhouses none of the listed criteria for allocation of an animal to the sample population such as age, production type, breed, sex, farm of origin, canton of origin, vaccination status and breeding farm, was considered to be reliably and timely detectable with the given infrastructure and data management systems.

This difficulty of selecting animals according to the listed criteria was also reflected in the compliance rate of the final sample. The evaluation of the survey sample based on information stored in the AMD revealed that from 3663 sampled animals 611 (16.7%) were older than 18 months and 15 (0.4%) younger than 6 months. Of the

1283 sampled farms, 71 (5.5%) had more than 6 animals in the sample. Forty-nine of these farms delivered cattle to more than one of the involved slaughterhouses and/or slaughtered at more than one day over the sampling period.

Discussion

The slaughterhouses included in this study differ from all others in size and capacity. Animals from all over the country end in one of the studied facilities as shown in Figure 1. However, the southern part of Switzerland is notably underrepresented. If routine surveillance is to be conducted in the slaughterhouses, some of the small slaughterhouses in underrepresented areas are to be included in the sampling scheme to assure geographical representativeness of the sample.

The feasibility study showed that all considered sampling matrices could basically be gained in the slaughterhouses. However, only blood, muscle meat and red organs are suitable for large scale sampling. Blood and meat juice are the preferred sampling matrices for routine surveillance programmes, although the diagnostic tools for meat juice for many relevant bovine pathogens do not yet exist. Thus, there is little practical experience with sampling. Nevertheless, taking meat samples from the carcass is a simple procedure with several advantages. The carcass is identifiable at every stage of the slaughter line and has by far the largest time window for sampling. It is either possible to take the sample during meat inspection or on the way between weighing and the chill rooms. If necessary, missed samples can be recovered (in most slaughterhouses) in the chill rooms, even hours after the animal has entered the slaughtering process.

The identification of animals according to sampling criteria is for the time being the limiting factor for better exploiting the slaughterhouses for the surveillance of the

cattle population. For instance, in many mandatory monitoring programmes, the herd is the unit of interest and several animals per herd have to be sampled. In Switzerland, cattle are gathered by traders on the farms and often only a few or single animals per farm are collected and delivered at the slaughterhouse. The distribution of animals to the slaughterhouses is also economically driven, as slaughterhouses have different preferences for full-meat or lean animals. So animals of one farm are slaughtered on scattered days and often in different abattoirs (Figure 2). Additionally, the animals are not consistently announced to the slaughterhouses before their delivery and therefore the slaughterhouses cannot plan the sampling ahead.

The pilot study revealed that animals are not easily distinguishable by production type. The sample selection based on matching with the databases (AMD or slaughterhouse intern) after the rough pre-selection at the slaughter chain was inefficient. Personnel resources and sampling material were wasted since too many samples had to be discarded due to the poor pre-selection of suitable animals.

The scenarios prepared for the workshop revealed that if only farms were sampled that delivered a certain number of animals on one day to one slaughterhouse, the sampling period would be considerably prolonged to reach a given number of sampled farms and animals (Figure 2a). Short sampling periods are typically applied in cross-sectional studies to estimate prevalence and to substantiate freedom from diseases. However, for studies aiming at early detection of emerging or re-emerging diseases, continuous sampling would be more appropriate. Furthermore, to assure that only animals from farms are sampled that deliver a certain minimum number of animals, a high logistic effort in the slaughterhouses is necessary, which is difficult to accomplish. It is impossible to avoid double sampling of farms that slaughter on

several days or in different slaughterhouses twice or more during the sampling period.

In addition, targeted sampling based on criteria on animal level is only achievable to a limited degree. Age categories, farm of origin, production type, breed and even the sex of the animals are difficult if not impossible to recognize at most of the positions relevant for sampling along the slaughter chain. Only the differentiation between calves and older animals is visually easily feasible, although not precise (calves can vary in age, weight and size). There was a general consent among the slaughterhouse veterinarians that the samples should be taken by qualified personnel (e.g. official veterinarian or meat inspectors). Moreover, most mandatory monitoring programmes require samples to be taken by official veterinarians or meat inspectors. This implies that the samples have to be taken either on a position in the slaughter chain where someone qualified is already present, or someone has to be specifically employed for the sampling. In the latter case, the number of samples per day should be large and concentrated enough to be economically reasonable and to justify the employment of an additional person.

If animals have to be selected according to given criteria, the suitable animals are most likely scattered over time and the sampling is only economically reasonable if it can be conducted during meat inspection. This is particularly important for constant surveillance applied during control programmes.

In conclusion, the relocation of the sampling of the cattle population to the slaughterhouses is possible. However, without a centralized data management system connected to the data management system of the slaughterhouse enabling real–time, or near real-time, data exchange, and without technical aids such as visual

signals to identify preselected animals, the possibilities of risk-based or even herd-level sampling are very limited.

The slaughterhouse visits showed that it was very difficult to appraise expenses for sampling without specifying the sample volume, the sampling time frame and the sampling material. The collaboration with slaughterhouse personnel at every stage of the planning and implementation of surveillance programs in a slaughterhouse is important to assure compliance and avoid unreasonable or even unfeasible specification for sampling. The resources required for implementing routine monitoring and surveillance in slaughterhouses needs to be carefully assessed.

Practical importance

Some countries have sophisticated data management systems combined with audio-visual aids in place to timely identify cattle for targeted sampling in slaughterhouses. But to our knowledge, feasibility of sampling targeted (predetermined) animals in slaughterhouses with standard equipment has never been systematically assessed and published. Although based on Swiss data, our study provides useful knowledge and arguments for other countries considering exploiting cattle slaughterhouses for surveillance purposes more efficiently.

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References

1. ANONYMOUS (1995) Animal health ordinance (TSV), Art. 130. -2. ANONYMOUS (1999) Agreement of 21 June 1999 between the Swiss Confederation and the European Community on trade in agricultural products , Annexe 11, SR 0.916.026.81 - 3. BUNDESAMT FÜR VETERINÄRWESEN FVO (2012). Blauzungen-Überwachung in der Schweiz, Erklärung der Seuchenfreiheit. Internet publication. - 4. CORBELLINI LG, SCHWERMER H, PRESI P, THÜR B, STÄRK KD, REIST M (2006). Analysis of national serological surveys for the documentation of freedom from porcine reproductive and respiratory syndrome in Switzerland. Vet Microbiol. 118:267-73. – 5. GOHM, DS, AUDIGE, L, HOFMANN, MA (1999): A survey of Newcastle disease in Swiss laying-hen flocks using serological testing and simulation modelling. Prev Vet Med. 38/4:277-88. – 6. HADORN DC, RACLOZ V, SCHWERMER H, STÄRK KD (2009). Establishing a cost-effective national surveillance system for Bluetongue using scenario tree modelling. Vet Res. 40:57. – 7. R DEVELOPMENT CORE TEAM (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/ - 8. REBER A, REIST M, SCHWERMER HP (2012). Cost-effectiveness of bulk-tank milk testing for surveysto demonstrate freedom from infectious bovine rhinotracheitisand bovine enzootic leucosis in Switzerland. SAT 154 Heft 5: 189-197. – 9. REIST M, JEMMI T, STÄRK K (2012). Policy-driven developement of cost-effective, risk-based surveillance strategies. Prev Vet Med. Jul 1; 105 (3): 176-184. – 10 SQUIRREL SQL CLIENT, URL http://www.squirrelsql.org/ - 11. STÄRK KD, REGULA G, HERNANDEZ J, KNOPF L, FUCHS K, MORRIS RS, DAVIES P (2006). Concepts for risk-based surveillance in the field of veterinary medicine and veterinary public health: review of current approaches. BMC

Health Serv Res. 6:20.- 12. THE POSTGRESQL GLOBAL DEVELOPMENT GROUP (2013), URL http://www.postgresql.org/

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Table 1: Possible sampling matrices according to process stage in the slaughter line. Process stages where sampling was generally not considered practicable are excluded (stunning, shackling, hoisting, pre-dehiding steps, brisket sawing, evisceration, removal of thoracic organs, carcass splitting). *Italic:* sampling possible but unpractical. **Bold**: preferred matrices and process stage for large scale sampling.

Process stage in the slaughter line	**Possible sampling matrices in all study slaughterhouses**	**Possible sampling matrices in some study slaughterhouses**
unloading/ chute	*blood, skin, hair, nose/laryngeal/conjunctival swab, faeces*	
lairage		
ante-mortem inspection		
bleeding	**blood**	skin, hair, nose/laryngeal/conjunctival swab, faeces
fore-foot removal		skin, hair
head removal		skin, hair, nose/laryngeal/conjunctival swab, CNS
dehiding		skin, hair
post-mortem inspection	**blood**, **muscle meat, red organs**, **modified or suspect tissues/organs** faeces, white organs	skin, hair, nose/laryngeal/conjunctival swab, CNS
processing room for tripe and other organs		skin, hair, nose/laryngeal/conjunctival swab, faeces, red organs, white organs, modified or suspect tissues/organs
spinal cord removal	**Tissues of the Central Nervous System**	
carcass grading/weighing	**muscle meat**	
carcass chilling		muscle meat

Table 2: Sampling scenarios for the workshop held with slaughterhouse veterinarians and stakeholders from the official veterinary service to discuss the results from the preceding slaughterhouse visits. The resulting number of samples taken and duration of each sampling scenario (columns on the right) were calculated with R based on real Swiss slaughter data (AMD) of the year 2009. The sampling population is defined as beef cattle older than six months for all scenarios.

Scenario	Sampling criteria Animals to be sampled	Central data management system necessary	No. of samples	Max no. of samples/day/ slaughterhouse	Duration (months)
I	**All** suitable (beef production, not calves) animals	No.	13‘912	286	~3
II	Exactly five animals from beef farms that deliver at least five animals on a single day to the given slaughterhouse.	No	22‘280	115	~6
III	Exactly five animals from beef farms that deliver at least five animals on a single day to the given slaughterhouse and that have not yet been sampled in the **same** slaughterhouse.	No	9‘400	70	~6
IV	Exactly five animals from beef farms that deliver at least five animals on a single day to the given slaughterhouse and that have not yet been sampled in **any** slaughterhouse.	Yes	7‘515	70	~6
V	Like for Scenario IV Sample no. **limited to 20** per day and slaughterhouse.	Yes.	7‘500	20	~12

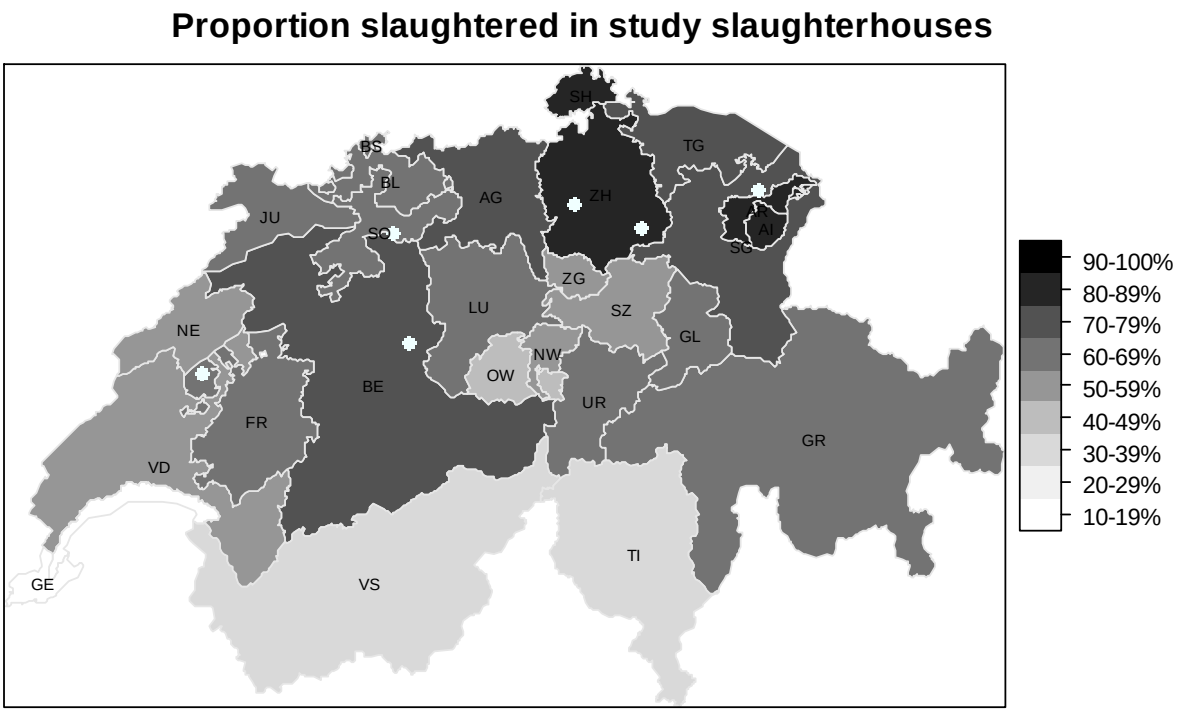

Figure 1: Proportion of cattle slaughtered in the six biggest slaughterhouses in Switzerland per canton. White dots: study slaughterhouses.

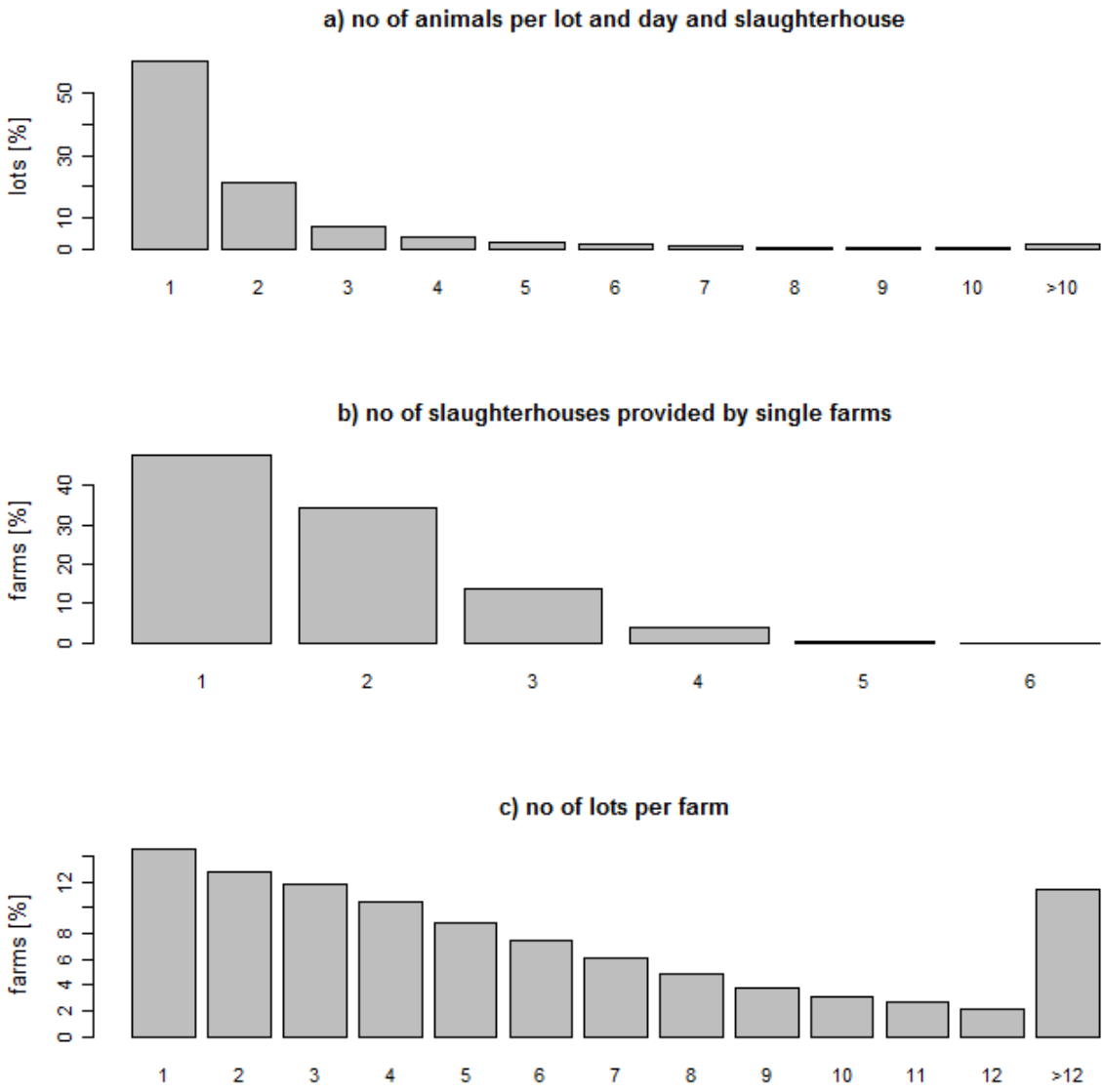

Figure 2: Slaughter practice of Swiss farmers in 2009. A lot is defined as the number of animals that are delivered from one farm to one of the study slaughterhouses on one day. a) Number of animals per lot, slaughterhouse and day from one farm. b) Number of study slaughterhouses supplied by a single farm with at least one lot. c) Number of lots one farm brought to the study slaughterhouses over the year.

9 Extended spectrum beta-lactamase producing *Enterobacteriaceae*: occurrence, risk factors for fecal carriage and strain characteristics in the Swiss cattle population younger than 2 years sampled at slaughterhouse level

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Short title: Prevalence of ESBL producing *E. coli* in cattle at slaughter

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SUMMARY

During the past decade extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* have become a matter of great concern in human and veterinary medicine. In this cross-sectional study fecal swabs of a geographically representative number of Swiss cattle at slaughterhouse level were sampled i) to determine the occurrence of ESBL producing *Enterobacteriaceae* in the Swiss cattle population younger than 2 years, and ii) to assess risk factors for shedding ESBL producing *Enterobacteriaceae*. In total, 48 (8.4%; 95% C.I. 6.3 – 11.1%) independent ESBL producing *Enterobacteriaceae* were detected among the 571 tested animals. Species identification revealed 46 *E. coli* strains, one *Enterobacter cloacae* and one *Citrobacter youngae.* In view of β-lactam antibiotics, all 48 isolates were resistant to ampicillin, cephalothin and cefpodoxime. Forty-five (93.8%) isolates were resistant cefuroxime; one (2.1%) isolate to cefoxitin, 28 (58.3%) isolates to cefotaxime, 2 (4.2%) isolates to ceftazidime, and 2 (4.2%) isolates to cefepime. Risk factors for shedding ESBL producing *Enterobacteriaceae* were (i) age (OR 0.33 and 0.22 in age category 181 d to 1y and 1y to 2 y compared to ≤ 180 d), (ii) primary production type, meaning dairy compared to beef on farm of origin (OR 3.11), and (iii) more than 1 compared to less than 1 animal movement per d per 100 animals on farm of origin (OR 2.06).

Keywords: *E. coli*, ESBL, cattle, slaughterhouse

INTRODUCTION

Antimicrobial resistance in bacteria has emerged as a problem in both human and veterinary medicine. One of the currently most important resistance mechanisms in *Enterbacteriaceae*, which reduces the efficacy even of modern expanded-spectrum cephalosporins (except cephamycins and carbapenems) and monobactams is based on plasmid-mediated production of enzymes that inactivate these compounds by hydrolyzing their β-lactam ring. Such resistance is encoded by an increasing number of different point-mutational variants, called extended spectrum β-lactamases (ESBL), of classical broad-spectrum β-lactamases (BSBL): most are derivates of TEM and SHV β-lactamase families, whereas other groups, such as CTX-M, OXA, PER and VEB β-lactamases have been described more recently (1). The phenotypical difference between BSBLs and ESBLs is that the latter efficiently hydrolyze 3rd and 4th-generation cephalosporins, additionally to penicillins and lower generation cephalosporins as the BSBLs are capable of. ESBLs are inhibited by clavulanic acid, sulbactam and tazobactam (2), a feature that is used (i) as a criterion for classification of β- lactamases and (ii) for diagnostic ESBL detection purposes. As a matter of growing concern, resistance caused by ESBLs is often associated with resistance to other classes of antibiotics like fluoroquinolones, aminoglycosides and trimethoprim-sulfmethoxazole (3,4). Since the first description of ESBL producing *Enterobacteriaceae* isolated from hospitalized humans (5), many nosocomial outbreaks have been reported. However, since a few years, there is an increase in the detection of ESBL producing strains in the community (6,7). More recently, some reports have alerted about the dissemination of ESBL producing *E. coli* in healthy cattle in several countries in Europe and USA (8,9,10) or in cattle derived food products like meat and raw milk (11,12,13). Therefore, the impact of healthy farm animals as a reservoir for an input of ESBL producing *E. coli* in the food processing chain has to be assessed. The aim of the present study was to assess the prevalence of ESBL producing *Enterobacteriaceae* in the Swiss cattle po 75

pulation younger than two years, and to assess risk factors for shedding ESBL producing *Enterobacteriaceae* based on a slaughterhouse monitoring approach that is aiming at achieving a geographically representative sample.

METHODS

Sampling

Representative samples for the cattle population younger than 2 years were taken at the slaughterhouse level. A minimum required sample size of 385 randomly selected animals was calculated with the assumptions of an infinite population size, a prevalence of 50%, a desired confidence level of 95.0% and an absolute error of 5% (Win Episcope 2.0 software http://www.clive.ed.ac.uk/winepiscope). The samples were randomly taken at the five biggest cattle abattoirs (A to E), where over 75% of Swiss cattle of the targeted age group are slaughtered. Because animals originating from farms located south of the Alps are typically underrepresented in the slaughter population of these 5 biggest abattoirs, samples from the biggest abattoir in the canton of Ticino (F) and samples from several smaller abattoirs from the canton of Valais were additionally collected to guarantee a geographically representative distribution. Only one sample was taken per animal holding of origin. The number of samples in the sampling frame collected from each slaughterhouse was proportional to the number of cattle slaughtered at each establishment per year. Based on these data, a random sampling plan was conceived. Faecal samples were collected from November 2010 to September 2011 from 571 healthy animals at slaughter. The samples were taken through opening the large intestine with sterile scissors after evisceration. Unique animal identification numbers registered in the Swiss central animal movement database (AMD) were recorded.

Microbiological analysis

Each sample was incubated for 24 hours at 37 °C in EE Broth (BD, Franklin Lakes, USA) for enrichment. The enriched faecal samples were inoculated onto Brilliance ESBL agar (Oxoid, Hampshire, UK) and incubated at 37 °C for 24 hours under aerobic conditions. All grown colonies were selected and sub-cultured onto Triple Sugar Iron (TSI) agar (BD, Franklin Lakes, USA) at 37 °C for 24 hours. By the oxidase test and the assessment of lactose fermentation, non-fermenters were discarded, and oxidase-negative colonies were subjected to identification by API ID 32 E (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing and ESBL detection

All isolated strains were subjected to susceptibility testing for 18 antimicrobial agents by the disc diffusion method and evaluated according to CLSI criteria (14). Strains exhibiting intermediate resistance were classified as susceptible. The antibiotics tested were: ampicillin (AM), amoxicillin/clavulanic acid (AMC), cephalothin (CF), cefuroxime (CXM), cefoxitin (FOX), cefpodoxime (CPD), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), ciprofloxacin (CIP), nalidixic acid (NA), gentamicin (GM), streptomycin (S), trimethoprim-sulfamethoxazole (SXT), chloramphenicol (C) and tetracycline (TE). The amoxicillin/clavulanic acid disc was placed between the cefpodoxime and the ceftazidime discs, and the synergy effect was documented. The strains, which showed a synergy effect, were then confirmed as ESBL producers on Muller-Hinton agar plates using E-Test-ESBL strips containing cefotaxime, cefepime or ceftazidime alone and in combination with clavulanic acid (bioMérieux, Marcy l'Etoile, France).

Risk Factor Analysis

Risk factors were calculated based on data obtained from the Swiss AMD. The individual animal identification number of the slaughtered and sampled animals served as a unique identifier to determine the farm of origin, date of birth and to access all movement data

corresponding to the farm of origin and the 127 length of stay of each individual animal of the sample. Risk factors derived from AMD data comprised age, main production type on the farm of origin (dairy vs. beef and fattening), farm size, number of animal movements and number of animals found death or euthanized on farm. The full list of studied risk factors and their categorization is given in Table 1.

Statistical Analysis

Statistical analyses were performed in R version 2.13 for Mac OS. The significance level was set at $p \leq 0.05$. ESBL prevalence and their Yates' continuity corrected 95% confidence intervals were calculated applying exact binomial tests. Logistic regression models were applied for risk factor analyses. First, a univariate model was calculated for each risk factor given in Table 1. Risk factors with a p-value < 0.25 were retained for multivariate analyses. To avoid colinearity, the correlation structure of these retained risk factors was assessed. Of each pair of correlating risk factors, only the one showing the more significant association with the dependent variable was retained for the multivariate model. Multivariate models were fitted by backward elimination procedures. According to Hosmer and Lemeshow (15) confounders were eliminated if they did not importantly change the estimates of the significant predictors. All two ways interactions were tested.

RESULTS

The sampling regimen resulted in a geographically representative sample population that was uniformly spread over the entire territory of Switzerland. Farms of origin of cattle shedding ESBL-producing Enterobacteriaceae are as well uniformly spread over the densely populated Swiss midlands as well as over the canton of Ticino in the south east of Switzerland. There were no cattle shedding ESBL-producing Enterobacteriaceae found originating from the canton of Valais in the south west of Switzerland.

ESBL-producing Enterobacteriaceae were detected in 48 (8.4%; 95% C.I. 6.3–11.1%) of the 571 tested animals. ESBL prevalence in different age classes, different production types and different animal movement activities in the farm of origin are summarized in Table 2. Results of the final multivariate logistic regression models applied for risk factor analysis are shown in Tables 3 and 4. Animals from within the age class ≤180 days were at a significantly higher risk of shedding ESBL-producing Enterobacteriaceae than animals from age classes 180 days to 1 year or 1 year to 2 years. Animals originating from farms with primary production type „dairy“ were at a 5.95 times greater risk of shedding ESBL-producing Enterobacteriaceae than animals originating from farms with primary production type „beef“. Finally, animals originating from farms with more than one animal movement per day per 100 animals were at a 2.37 times higher risk of shedding ESBL-producing *Enterobacteriaceae* than animals originating form farms with less than one animal movement per day100 animals.

DISCUSSION

In this cross-sectional study, fecal samples of a geographically representative cattle subpopulation for Switzerland were sampled at slaughterhouse level to determine the occurrence of ESBL producing *Enterobacteriaceae* in the Swiss cattle population younger than 2 years, and to assess risk factors for shedding such organisms. To assess the population prevalence of a pathogen on a national level, a geographically representative set of samples needs to be selected. Whilst this is easily achieved for samples taken on a farm level, where farms to be sampled can be determined in advance by applying a stratified randomization scheme, this is typically more difficult for samples taken at the slaughterhouse. This study demonstrated that the goal of a geographically representative survey could be achieved with a carefully planned sampling scheme involving the major cattle slaughterhouses and additional small abattoirs in areas disconnected from the main animal traffic. To our knowledge, this is the first study on risk factors for shedding ESBL producing *Enterobacteriaceae* working with risk factors derived from animal movement data stored in a national AMD. Performing risk factor analysis with such data offered several advantages compared to risk factors derived from surveys, especially when the samples were collected at the abattoir and not on the farm. Getting information on animal movements and mortalities from 571 farms of origin of slaughtered animals via telephone or email surveys would be very time consuming. Furthermore, such information would be very imprecise compared to data from the Swiss AMD whose content is very accurate and complete.

The overall prevalence of cattle hosting ESBL producing *Enterobacteriaceae* found in this study (8.4%; 95% C.I. 6.3 – 11.1%) is slightly lower than found in previous, smaller scaled Swiss studies that reported prevalences of 13.7% (16) and 17.1% (10). Recent studies from other countries reported a very low (0.2%) prevalence in cattle in Korea (17) or even zero prevalence in cattle in Tunisia (18). Studies from European countries reported 35.4% (22.2 – 50.5 % C.I. 95%) in North West England and North Wales (19)

and 4.8% in France (20). One of the significant risk factors for shedding ESBL producing *Enterobacteriaceae* obtained in the final multivariate model was age, with cattle over 6 months being at a considerably lower risk than calves (younger than 6 months) This is in accordance with a previous study of Geser et al. (16) who reported a overall prevalence of 13.7% in cattle as opposed to a prevalence of 25.3% among calves. Animals from dairy farms were at a 3.1 times higher risk for hosting ESBL producing *Enterobacteriaceae* than animals from beef or fattening farms. This might be explained by differences between production types with respect to farming practices and antimicrobial compounds applied. In Switzerland, sales of cephalosporins have increased over the past years, especially the sales of 3rd and 4th generation cephalosporins for intramammary application (21). Snow et al. (19) reported that in North West England and North Wales farms that had used 3rd or 4th generation cephalosporins in livestock during the previous 12 months were nearly 4 times more likely to host ESBL *E. coli*. On Swiss dairy farms, calves not in consideration for breeding are either fattened on their farm of birth or they are sold to fattening farms at a very young age. Those fattened on the dairy farms are primarily fed with milk. For economic reasons, milk that cannot be put on the market because of elevated cell counts or because of recent antimicrobial treatments is often fed to calves. Moreover, ESBL hosting *Enterobacteriaceae* present on dairy farms might be transmitted to calves by the fecal-oral route. Calves later to be fattened on fattening farms leave their dairy farms very few weeks after birth and are thus at a lower risk for acquiring ESBL producing *Enterobacteriaceae* than cows staying on the dairy farms until slaughter. In comparison to dairy farms, β-lactam antibiotics, especially 3rd and 4th generation cephalosporins, do not represent the predominantly used antimicrobial compounds on beef and fattening farms. The fact that among 40 animals originating from the canton of Valais no ESBL producers were found might also be explained by the farming type, as meat production is predominant over milk production in this area. The number of animal movements per

farm per day per 100 animals is a factor related to introduction of new stock, and it was associated with presence of ESBL *Enterobacteriaceae*. Animals from farms with a high number of animal movements in relation to total farm size were at a 2 times higher risk of hosting ESBL producing *Enterobacteriaceae* than animals from farms with a lower level of animal traffic. This is in agreement with Snow et al. (19) who reported that operating a closed farm policy reduced the risk of the farm having ESBL *E. coli* compared to farms that were open and did not quarantine new cattle.

This study showed that with a carefully planned sampling scheme involving the major cattle slaughterhouses and – in addition – small abattoirs in areas disconnected from the main animal traffic, geographically representative surveys can be achieved by taking random samples at the abattoir. The confirmation of relatively high rates of ESBL producers in cattle and the high diversity among the isolates are worrisome and indicate an established reservoir, especially in dairy farms. A more prudent use of antibiotics, especially of 3rd and 4th generation cephalosporins, restrictions in feeding milk of treated cows to calves and management improvements to facilitate the operation of closed herd policies could represent modes of action towards reduction of ESBL prevalence in cattle. Experimental studies would be needed to assess the effectiveness of such measures.

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REFERENCES

1. Coque TM, Baquero F, Cantòn R. 2008. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Eurosurveill. **13** (47) pi:19044.

2. Bradford PA. 2001. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. **14**:933-951.

3. Cantòn R, Coque TM. 2006. The CTX-M β-lactamase pandemic. Curr. Opin. Microbiol. 276 **9**:466-475.

4. Gniadkowski M. 2001. Evolution and epidemiology of extended-spectrum beta lactamases (ESBLs) and ESBL-producing microorganisms. Clin. Microbiol. Infect. 279 **7:**597-608.

5. Knothe H, Shah 280 **P, Krcmery V, Antal M, Mitsuhashi S.** 1983. Transferable resistance to cefotaxime, cefoxitin, cefamamdole and defuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infect. **11**:317-317.

6. Mesa RJ, Blanc V, Blanch AR, Cortés P, González JJ, Lavilla S, Miró E, Muniesa M, Saco M, Tórtola MT, Mirelis B, Coll P, Llagostera M, Prats G, Navarro F. 2006. Extended-spectrum b-lactamase-producing *Enterobacteriaceae* in different environments (humans, food, animal farms and sewage). J. Antimicrob. Chemother. **58**:211-215.

7. Geser N, Stephan R, Korczak BM, Beutin L, Hächler H. 2012. Molecular identification of extended-spectrum-β-lactamase genes from *Enterobacteriaceae* isolated from healthy human carriers in Switzerland. Antimicrob. Agents Chemother. **56**:1609-1612.

8. Meunier D, Jouy E, Lazizzera C, Kobisch M, Madec JY. 2006. CTX-M-1- and CTX-M- 15-type beta-lactamases in clinical *Escherichia coli* isolates recovered from food293 producing animals in France. Int. J. Antimicrob. Agents **28**:402-407.

9. Wittum TE, Mollenkopf DF, Daniels JB, Parkinson AE, Mathews JL, Fry PR, Abley MJ, Gebreyes WA. 2010. CTX-M-type expended-spectrum beta-lactamases

present in *Escherichia coli* from the feces of cattle in Ohio, United States. Foodborne Path. Dis. **7**:1575-1579.

10. Geser N, Stephan R, Kuhnert P, Zbinden R, Kaeppeli U, Cernela N, Haechler H. 2011. Fecal carriage of extended-spectrum ß-lactamase-producing *Enterobacteriaceae* in swine and cattle at slaughter in Switzerland. J. Food Prot. **74**:446-449.

11. Jensen LB, Hasman H, Agersø Y, Emborg HD, Aarestrup FM. 2006. First description of an oxyimino-cephalosporin-resistant, ESBL-carrying *Escherichia coli* isolated from meat sold in Denmark. J. Antimicrob. Chemother. **57**:793-794.

12. Hammad AM, Ahmed AM, Ishida Y, Shimamoto T. 2008. First characterization and emergence of SHV-60 in raw milk of a healthy cow in Japan. J. Vet. Med. Sci. **70**:1269-1272.

13. Doi Y, Paterson DL, Egea P, Pascual A, López-Cerero L, Navarro MD, Adams-Haduch JM, Qureshi ZA, Sidjabat HE, Rodríguez-Baño J. 2010. Extended spectrum and CMY-type beta-lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville, Spain. Clin. Microbiol. Infect*.* **16**:33- 38.

14. CLSI. Clinical 312 and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. CLSI Document M100-S18 2008, Wayne, PA.

15. Hosmer DW, Lemeshow S. 1989. Applied logistic regression. Wiley and Sons, New York, NY, USA.

16. Geser N, Stephan R, Hächler H. 2012. Occurrence and characteristics of extended318

spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* in food producing animals, minced meat and raw milk. BMC Vet. Res. **8**:21.

17. Tamang MD, Nam HM, Kim SR, Chae MH, Jang GC, Jung SC, Lim SK**. 2013.** Prevalence and molecular characterization of CTX-M β-lactamase-producing *Escherichia coli* isolated from healthy swine and cattle. Foodborne Path. Dis. 2013; **10**:13-20.

18. Ben Sallem R, Ben Slama K, Sáenz Y, Rojo-Bezares B, Estepa V, Jouini A, Gharsa H, Klibi N, Boudabous A, Torres C. 2012. Prevalence and characterization of extended-spectrum beta-lactamase (ESBL)- and CMY-2-producing *Escherichia coli* Isolates from healthy food-producing animals in Tunisia. Foodborne Path. Dis. **9**:1137-1142.

19. Snow LC, Warner RG, Cheney T, Wearing H, Stokes M, Harris K, Teale CJ, Coldham NG. 2012. Risk factors associated with extended spectrum beta-lactame *Escherichia coli* (CTX-M) on dairy farms in North West England and North Wales. Prevent. Vet. Med. **106**: 225-234.

20. Hartmann A, Locatelli A, Amoureux L, Depret G, Jolivet C, Gueneau E, Neuwirth C. 2012. Occurrence of CTX-M producing *Escherichia coli* in soils, cattle, and farm environment in France (Burgundy Region). Front. Microbiol. **3**:83.

21. Anonymous ARCH-VET 2011: Swiss annual report on usage of antimicrobials in veterinary medicine and on monitoring antimicrobial resistance in food animals. (http://www.swissmedic.ch/marktueberwachung/00147/00644/index.html?lang=de). Accessed 03-10-2013.

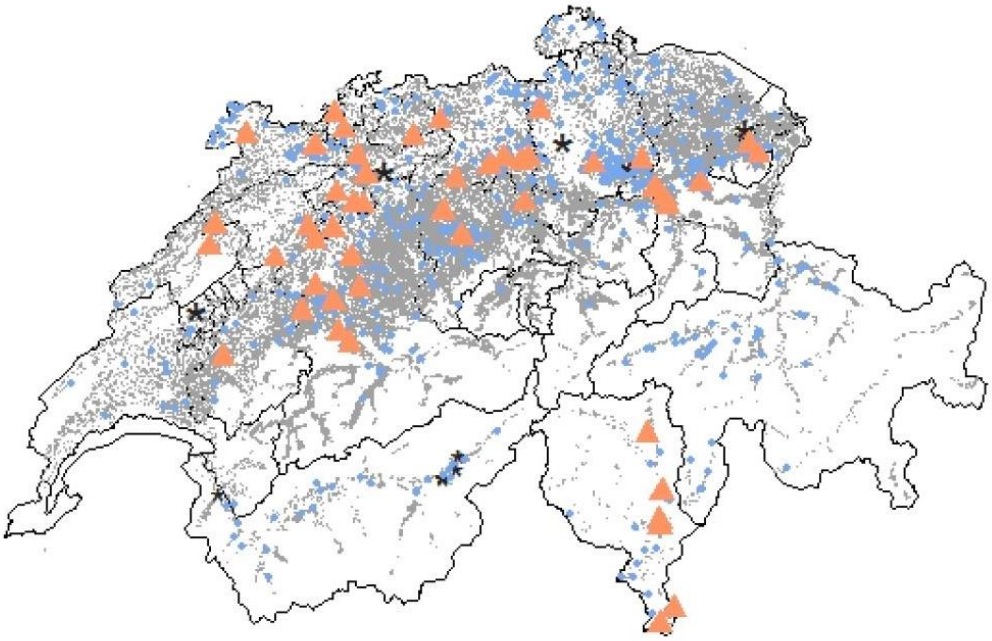
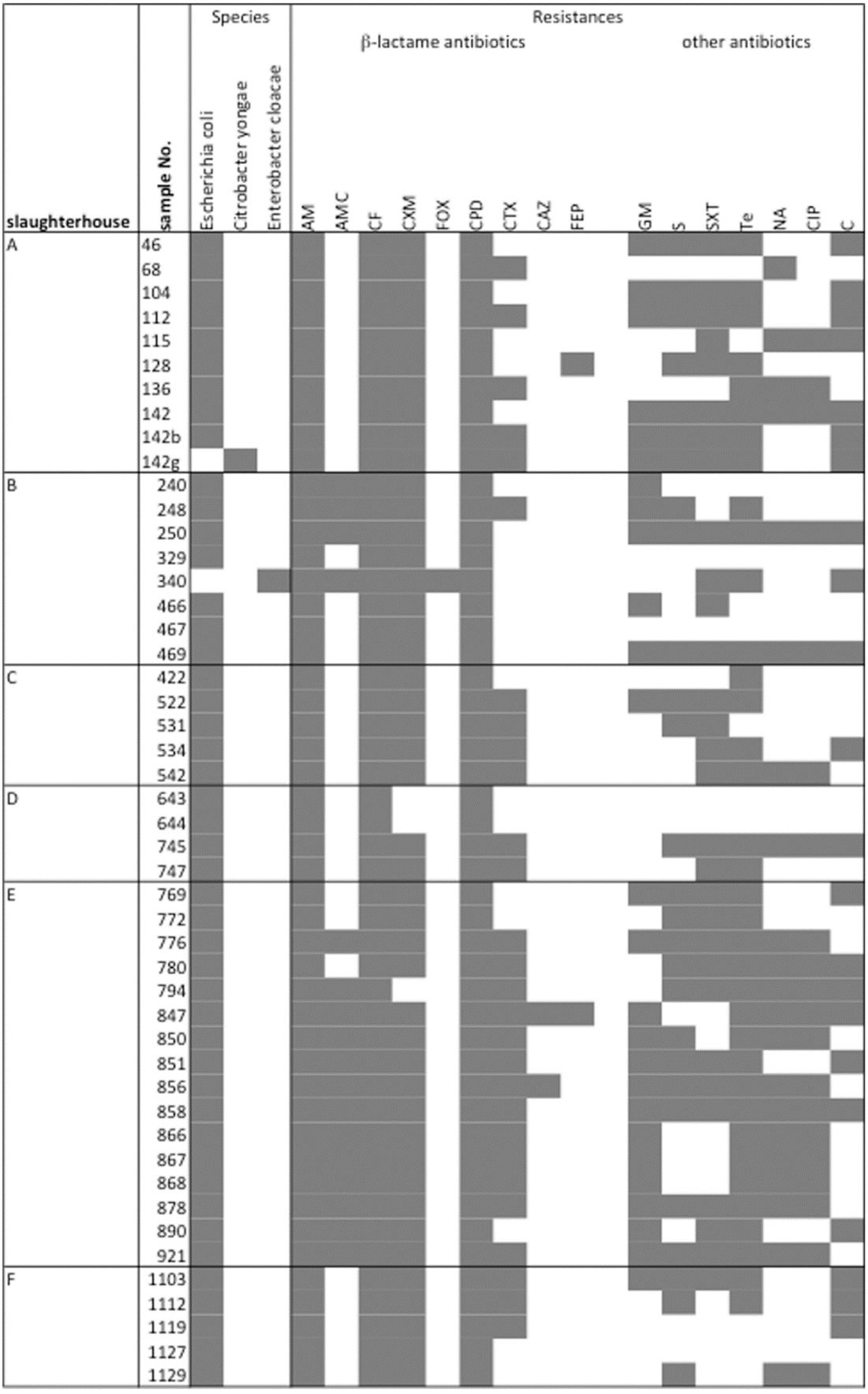


Figure 1: Geographical distribution of all Swiss cattle holdings hosting animals ≤ 2 years (grey background), distribution of the farms of origin of the sample population (blue circles), distribution of farms of origin of cattle shedding ESBL-producing *Enterobacteriaceae* (orange triangles) and the distribution of the participating slaughterhouses (black stars) are depicted.


Species
Resistances
β-lactame antibiotics
other antibiotics
slaughterhouse
sample No.
Escherichia coli
Citrobacter yongae
Enterobacter cloacae
AM
AMC
CF
CXM
FOX
CPD
CTX
CAZ
FEP
GM
S
SXT
Te
NA
CIP
C
A
46
68
104
112
115
128
136
142
142b
142g
B
240
248
250
329
340
466
467
469
C
422
522
531
534
542
D
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644
745
747
E
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1127
1129

Figure 2: Characteristics and antimicrobial susceptibility profiles of ESBL-producing *Enterobacteriaceae* isolated from cattle younger than 2 years. Symbols: black square, positive result or resistant to a specific antimicrobial agent; white square, negative result or susceptible to a specific antimicrobial. Abbreviations: AM, ampicillin; AMC, amoxicillin-clavulanic acid; CF, cephalothin; CXM, cefuroxime; FOX, cefoxitin; CPD, cefpodoxime; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; GM, gentamicin; S, streptomycin; trimethoprim-sulfamethoxazole (SXT); TE, tetracycline; NA, nalidixic acid; CIP, ciprofloxacin; C, chloramphenicol. Discrimination between "susceptible" and "resistant" was strictly according to CLSI interpretive criteria. It should be noted, however, that for clinical and therapeutic purposes, ESBL producers should generally be reported resistant to cephalosporins of all 4 generations and monobactams.

Table 1: Description of risk factors studied in univariate and multivariate logistic regression models

Risk factor	Category	Number of animals
Age Class	≤ 180 d	248
	181d-1y	148
	≥ 1y	175
Production Type	Meat	244
	Dairy	327
Number of cattle on farm	≤30	173
	31-60	211
	≥60	187
Animal movements to farm per day	≤ 0.5	428
	> 0.5	143
Animal movements to farm per day per 100 animals	≤ 1 / d / 100 animals	461
	> 1 / d / 100 animals	110
Number of animals dying on farm per 100 days	0	235
	≤ 1 / 100 d	185
	> 1 / 100 d	151
Number of animals dying on farm per 100 days per 100 animals	0	235
	≤ 2 / 100 d	200
	> 2 / 100 d	136

Table 2. Prevalences of animals with ESBL positive isolates

	No. positives	No. negatives	prevalence	95% confidence interval *
Total	48	523	8.41%	6.32 – 11.07%
≤ 180 d	39	209	15.73%	11.55 – 21.00%
181 d – 1 y	5	143	3.38%	1.25 – 8.12%
> 1 y	4	171	2.29%	0.73 – 6.12%
Meat	6	238	2.46%	1.00 – 5.53%
Milk	42	285	12.84%	9.51 – 17.08%
≤ 1 mv. / d / 100 animals	32	429	6.94%	4.87 – 9.76%
> 1 mv. / d / 100 animals	16	94	14.55%	8.80 – 22.85%

* 95% confidence interval with Yates' continuity correction

Table 3. Farm level risk factors for ESBL shedding

Risk factor		OR	95% Conf. Int.	p-value
Prod. type	meat	1		
	dairy	3.11	1.18 - 8.21	0.02225
Movements	≤ 1 / d / 100 anim.	1		
	> 1 / d / 100 anim.	2.06	1.05 - 4.05	0.03461

Multivariate logistic regression model

Table 4. Animal level risk factors for ESBL shedding

Risk factor		OR	95% Conf. Int.	p-value
Age	≤ 180 d	1		
	181 d – 1 y	0.33	0.12 - 0.93	0.03597
	> 1 y	0.22	0.07 - 0.65	0.00663

Multivariate logistic regression model

10 Cost and sensitivity of on-farm versus slaughterhouse surveys for prevalence estimation and substantiating freedom from disease

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Abstract

Within the framework of Swiss surveillance for epizootic diseases, dairy cattle are sampled using bulk tank milk while non-dairy cattle are sampled on the farm. The latter method is costly, time-demanding and dangerous for the personnel. However, slaughterhouses could be an alternative sampling point for this population.

To assess the cost-effectiveness and sensitivity of such an approach, surveillance using slaughterhouse sampling was modelled with data from the 2012 Swiss animal movement database (AMD). We simulated a cross-sectional study for bluetongue (BT), and surveillance programmes to substantiate freedom from infectious bovine rhinotracheitis (IBR) and enzootic bovine leucosis (EBL) (combined) to compare the outcome of random on-farm sampling versus slaughterhouse sampling.

We found that, under Swiss conditions, slaughterhouse sampling results in low herd-level sensitivities because animals are sent by owners to slaughter individually and not in large groups, restricting the number of samples per herd. This makes slaughterhouse sampling inappropriate for prevalence surveys at the herd-level. However, for prevalence surveys at the animal- level and for substantiation of freedom from disease, slaughterhouse surveillance is equally or more cost-efficient than on-farm sampling.

Keywords: slaughterhouse surveillance, cross-sectional study, substantiate freedom from disease, simulation, animal movement database

Introduction

The Swiss cattle industry consists of small scale, predominantly dairy, farms.

The veterinary services invest substantial resources in surveillance to monitor cattle health. In 2010, sampling of dairy cattle was changed from on-farm sampling by individual blood samples to more cost-effective bulk tank milk testing (Reber et al., 2012; Reist et al., 2012; Schwermer et al., 2008). However, non-dairy farms, which represent one third of the cattle population, are still sampled on-farm. Blood sampling in suckler cow herds and specialised calf- and bull-fattening plants pose big challenges. Suckler cows are often kept under semi-free range conditions on alpine or pre-alpine pastures, at some distance from the farm, which makes them difficult, expensive and time-consuming to access. Furthermore, animals on non-dairy farms are usually not accustomed to being handled by humans, and are therefore difficult to tether and sample. Consequently, the risk of injuries is high for both the farmer and the veterinarian.

For these reasons, the Swiss Veterinary Service is currently considering a shift from on-farm sampling to slaughterhouse sampling for the routine surveillance of cattle. Slaughterhouse surveillance is not new; it has been implemented in many countries (Lynch and Silva, 2013). In Switzerland samples from pigs and poultry have been taken for many years at slaughter (Anonymous, 2014). However, in contrast to pigs and poultry cattle are rarely slaughtered in batches of origin from one farm. Usually, only small batches or single bovines from individual farms are slaughtered in one slaughterhouse on one day. Usually, cattle traders collect single bovines destined for slaughter from farms, organize batches and deliver them to the slaughterhouse on the same day (Schärrer et al., 2013). As a result, cattle from a single farm are slaughtered at several slaughterhouses per year. To get the best price at slaughter,

animals from one farm might end up in different slaughterhouses on the same day. Adult cows from calf suckler herds are slaughtered when they become unproductive. However, the farmer's decision to sell such animals to slaughter is likely to be influenced also by factors such as the availability of pastures, or the need to make room for new breeding stock. Slaughter may also be an alternative to treatment in case of a health event. These individual decisions add to the unpredictable pattern observed at the slaughterhouse.

As a result, it is not possible to predict when, where, or how many animals will be slaughtered from a particular farm. Additionally, to sample a group of bovines from the same farm to reach a certain herd-level sensitivity is especially complicated. Compared to on-farm blood sampling, this makes the planning of surveillance activities very challenging. The probability of detecting diseased herds in the population (i.e. the system sensitivity) depends on the herd-level sensitivity of each herd in the sample, which in turn is dependent on the number of animals sampled per herd (Cameron and Baldock, 1998; Christensen and Gardner, 2000; Martin et al., 1992). Contrary to on-farm sampling schemes, where sample size is determined based on the desired confidence level before the start of the surveillance programme (Schwermer et al., 2009), the number of sampled animals per herd and thus the number of herds to be sampled in a slaughterhouse sample is not straightforward to plan.

Over 600 slaughterhouses operate in Switzerland. For practical considerations, only the six largest cattle slaughterhouses are currently considered for inclusion in routine sampling. Together they cover about 70% of the cattle slaughtered. They all have enterprise resource planning (ERP) systems that could be adapted to transfer

information about the surveillance programmes to the meat inspectors (Schärrer et al., 2013). An IT solution for this information transfer is currently being developed.

The aim of this study was to compare the effectiveness (in terms of cost and sensitivity) of on-farm and slaughterhouse sampling for routine surveillance in Switzerland. Our approach was to simulate disease occurrence and surveillance scenarios on real demographic data extracted from the Swiss animal movement database (AMD). Two surveillance scenarios were simulated; 1) a prevalence survey for bluetongue (BT) – at the animal- and herd-level - and 2) a combined programme to prove freedom from infectious bovine rhinotracheitis (IBR) and enzootic bovine leucosis (EBL). A secondary aim was to estimate the geographical coverage and representativeness of a sample taken in the six biggest cattle slaughterhouses. The results of this study should support decisions regarding the shift from on-farm to slaughterhouse sampling in Switzerland.

Material and Methods

Data and data management

The AMD is the mandatory, nationwide registry for cattle in Switzerland. It contains records of all farms and all individuals, including all movements of live animals between farms from birth to death.

For the simulation models, data on all cattle registered as alive at least one day in 2012, and data on all slaughtered cattle in the same year, were extracted. Two data sets were used. The first dataset contained records of individual cattle with their bovine identification number (BID), farm identification number (FID) and dates of all between-farm movements. The second dataset contained BID, day of slaughter, the FID of the last farm the animal stayed on before slaughter and the ID of the slaughterhouse.

Available individual attributes included date of birth and sex. At the farm-level, the production type and the herd size of every farm on the first of January 2012 was extracted. If no animals were registered on a farm on this date, the herd size was set to 40, which corresponds to the average herd size.

The population on the first of January 2012 was described using summary statistics, as well as the slaughter population in the same year. The complex slaughter pattern in Switzerland was summarized as the distribution of slaughter lots in 2012. A slaughter lot was defined as the number of cattle slaughtered per day and slaughterhouse from the same farm. Only the six largest slaughterhouses were considered in this study.

Simulation of prevalence survey for BT

During the BTV-8 epidemic in northern Europe, three national vaccination campaigns against BT were conducted in Switzerland (Willgert et al., 2011). Because vaccinated animals remain seropositive for several years, serological surveillance must focus on animals born after the vaccination campaign. Currently BT surveillance in Switzerland is designed to prove freedom from disease at the animal level and samples are taken at the slaughterhouse; however, in this study we used BT as a model disease for a survey aiming to determine the prevalence at the herd level in the cattle population.

Diseased animals were introduced to the population by 'marking' individual bovines present in the population on 1st January 2012. The animals were marked in a two stage process. First, farms were randomly selected using the 'sample()' function in R (R Development Core Team, 2008), with a probability corresponding to the between-herd prevalence p^{bIni} (the number of herds in the sample was calculated as $p^{bIni} * H_0$, where H_0 is the number of herds in the population). Secondly, animals on these farms were attributed a zero (unmarked) or a one (marked) using a binomial distribution with a probability equal to the within-herd prevalence (p^{wIni}), which was set to 0.4 (Durand et al., 2010; Van Schaik et al., 2008). Between-herd prevalences (p^{bIni}) of 0.01, 0.1 and 0.5 were applied. Prevalence surveys for BT are conducted after the vector active period in Switzerland (Willgert et al., 2009) to reach a high sensitivity because the seroprevalence is highest at the end of the vector active period. Thus, transmission dynamics do not need to be considered in the simulation, because during the surveillance period no new infections occur.

In Switzerland, adult animals still have antibodies to BT, due to exposure or vaccination (Buchi et al., 2014). Therefore, only animals born after the end of the last

vaccination campaign in Switzerland (i.e. after 30th July 2010) and older than 6 months at the time of sampling (to avoid interference from maternal antibodies) are included in the surveillance. Consequently, the simulation was restricted to this population. We simulated a survey conducted during the vector-free period of 2012 (January-March). For the on-farm sample, 350 farms were randomly selected, and from every selected farm 10 animals that fulfilled the age criteria were included in the sample. The sample size was provided by the practice of the FSVO during the surveillance in 2008 to 2010. Every farm was randomly assigned to a sampling day (Monday-Friday) from 2 January to 31 March, 2012.

At the slaughterhouse, 60 cattle that fulfilled the age criteria were randomly selected per day and slaughterhouse, during the same time period as the on-farm survey. Without employing additional personnel for meat inspection a sample size of 60 specimens per day and slaughterhouse was considered feasible in a previous study (Schärrer et al., 2013).

The test sensitivity (Se_{Test}) and test specificity (Sp_{Test}) were set to 0.98 and 0.99 respectively. This is in line with a previous Swiss study that included two tests applied in the country (VMRD and PrioCHECK, personal communication), and where sensitivities between 97.3- 100% and specificities between 99.3 – 100% were estimated (Niedbalski, 2011). To account for an imperfect test, the marked animals in our data were assigned status as 'false negative' (FN) using a binomial distribution with probability 1- $Se_{Test..}$ A similar approach, but using (1-Sp_{Test}) was used to assign a "false positive" (FP) status to non-marked animals

The following outcome measures were calculated for the on-farm and slaughterhouse sample in every iteration (equations 1-8):

$$AP_i = \frac{n_i^+ - FN_i + FP_i}{n_i} \quad (21)$$

$$P_{herd} = \frac{\text{herds with positive animals } (n_i^+ > 0) \text{ in the sample}}{\text{herds in the sample}} \quad (22)$$

$$AP_{herd} = \frac{\text{herds classified positive}}{\text{herds in the sample}} \quad (23)$$

$$Se_{herd} = \frac{\text{herds correctly classified positive}}{\text{herds correctly classified positive + herds incorrectly classified negative}} \quad (24)$$

$$AP_{animal} = \frac{n^+ - FN + FP}{n} \quad (25)$$

where AP_i is the apparent within-herd prevalence of herd i, n_i^+ is the number of positive bovines in the sample from herd i, FN_i are the number of false negative animals and FP_i the number of false positive animals in the sample from herd i. P_{Herd} corresponds to the true herd level prevalence in the sample.

AP_{herd} is the apparent herd-level prevalence, where herds were classified as positive, if at least one positive animal (true or false positive) was detected.

Se_{herd} is the herd-level sensitivity of the survey. AP_{animal} is the apparent animal-level prevalence (n^+: positive bovines in the sample, FN: false negative bovines in the sample, FP: false positive bovines in the sample).

Table 1 shows the possible status combination for every animal in the simulation after the steps of marking, sampling and assignment of false negatives and positives. The proportions of marked animals and farms in the population during the period under study were affected by the removal of marked animals (through slaughter or natural death); the restocking of the population by calves born after the day of

marking (01.01.2012); or the moving of marked animals to a premise previously free of marked animals. The latter only affects the herd-level outcome.

For each p^{bini} the mean square error (MSE) of the herd level and animal level prevalence estimates was computed for the on-farm and for every day of the slaughterhouse sample. The MSE was used to compare the two sampling approaches in the simulation.

$$MSE = \frac{1}{r} * \sum_{1}^{r} \varepsilon_i^{\,2} \tag{26}$$

where r is the number of runs (1'000) and ε is $AP_{herd} - P_{herdpop}$ or $AP_{animal} - P_{pop}$. $P_{herdpop}$ and P_{pop} are the prevalence in the population after the marking on herd and animal level respectively. The lower the MSE, the more accurate is the sampling approach. The number of runs was chosen as test runs showed that larger numbers of iterations did not increase the precision of the model's outcome (see also the discussion on the introduced variation).

Figure 1 schematically represents the BT surveillance simulation model.

Simulation of surveillance to prove absence from EBL/IBR

Switzerland has been free from IBR and EBL since 1994 (Blickenstorfer et al., 2011). Bulk tank milk samples and blood samples collected on non-dairy farms are still tested yearly to substantiate freedom from both diseases. The requirement from the bilateral treaty with the EU is to show with 99 % confidence that less than 0.2 % of herds are infected (Anonymous, 2002).

We based the specification of the simulation of a survey to prove freedom from disease on the yearly surveillance programme for EBL/IBR. As the surveillance to prove the absence from disease is based on the assumption that no diseased animals are in the population, no fictional diseased bovines were introduced.

Dairy farms are covered by bulk tank milk testing, and therefore we focused on non-dairy farms only. In the surveillance programme of the Food Safety and Veterinary Office (FSVO), only cattle older than two years are sampled. We additionally simulated sampling of all cattle older than six months as a comparison.

For the on-farm sample, 2350 farms were selected at random and from every selected farm, a maximum of 50 animals that fulfilled the age criteria were included in the sample. The sample size and the limitation of 50 animals is current practice of the FSVO. For the scenario where only animals older than two years in which sampled, the sample was extended with younger bovines, if necessary to reach the sample size given by the FSVO. Every farm was randomly assigned to a sampling day (Monday-Friday) from 02.01.2012-29.02.2012.

Different sampling schemes were tested in the slaughterhouse to assess how the sensitivity of the programme could be maximized (minimum required sensitivity of 99%) and at what cost. To simulate two possible designs, the sample size was set a) to 60, assuming no additional personnel is available and b) to 200, to simulate a scenario where additional human resources are employed for sampling.

In the first scheme, random sampling at the slaughterhouse was assumed (Ran). To increase the herd-level sensitivity, a dynamic sampling scenario was tested (Dyn); after an initial random sample of 60 animals on day one, farms that were included in the sample from day 1 are given priority. The sample was then increased to achieve the given sample size, by random selection of additional animals. To refine the dynamic sample and to cover as many farms as possible, a scenario was defined in which a maximum of seven animals per farm was allowed (Lim). Finally, to simulate

a sampling scheme in which farm selection would be risk-based, the last scenario only sampled cattle from 5'000 defined, randomly selected farms (Fix).

The system sensitivity was calculated for the on-farm sample and for every day of sampling in the slaughterhouse. For the herd-level sensitivity (SeH_i) the following formulae were used (Sergeant, 2012):

If $\frac{n}{N_i} \leq 0.1 \quad SeH_i = 1-(1-Se_{test} * \frac{1}{N_i})^n$ (7.1)

if $\frac{n}{N_i} > 0.1 \quad SeH_i = 1-(1-Se_{test} * \frac{n}{N_i})$ (7.2)

if $\frac{n}{N_i} = 1 \quad SeH_i = 1-(1-Se_{test})$ (7.3)

where n is the number of animals in the sample, N_i is the herd size.

The system sensitivity (SSe) was calculated as follows (Sergeant, 2012):

if $\frac{h}{H_0} \leq 0.1 \quad SSe = 1-\Pi(1-SeH_i * p^{bini})$ (8.1)

if $\frac{h}{H_0} > 0.1 \quad SSe = 1-\Pi(1-\frac{\sum SeH_i}{h} * \frac{h}{H_0})^{p^{bini} * H_0}$ (8.2)

if $\frac{h}{H_0} = 1 \quad SSe = 1-(1-\frac{\sum SeH_i}{h})^{p^{bini} * H_0}$ (8.3)

were h is the number of herds in the sample and H_0 is the number of herds in the population. The number of runs was set to 1000.

Figure 2 schematically represents the simulation of the surveillance for freedom from disease.

Assigned costs

The cost of the surveillance programme for every run was calculated for on-farm and slaughterhouse sampling, respectively. The number of animals in the sample was multiplied with the sampling cost per animal, i.e. including time and material costs (7 € on-farm dairy, 9.95 € on-farm non-dairy and 5.8 € in the slaughterhouse; exchange rate CHF/€ = 1.2) and costs of laboratory analysis (17.4 €/sample). Travel expenses and work time for on-farm sampling where set at 20.7 and 29 € for dairy and non-dairy farms, respectively. The costs for on-farm sampling and laboratory analyses are based on a previous study on cost-effectiveness of sampling in Switzerland (Reist et al., 2012). The cost of a sample taken in a slaughterhouse is based on the price paid by the veterinary services per sample in slaughterhouse sampling surveys conducted during recent years.

The catchment area of the study slaughterhouses

To assess the geographical representativeness of the two sampling designs, cantons were chosen as the point of reference. Cantons are the regional government areas and each canton has an independent veterinary service. As in the current surveillance programmes, the on-farm sample was a-priori stratified by cantons, assigning numbers of farms to the sample proportional to the number of farms registered in the canton.

For the slaughterhouse sample for each canton the proportion of farms in the sample was calculated and compared to the proportion of registered farms. The difference in these proportions was used as measure for geographical representativeness.

Software

The simulations, analysis of the results and the graphical representation were undertaken using R (R Development Core Team, 2008)

Results

On 1st January 2012, 1'703'422 cattle were registered alive in the AMD. In the same year, 645'510 cattle were slaughtered (figure 3). Of the population alive on 1st January 2012, 683'024 cattle were born after 30th June 2010 and 445'787 were slaughtered in 2012 (designated 'BT' in figure 3). Slaughter lots are small for all studied scenarios (BT, IBR/EBL with animals older than six month and IBR/EBL older than two years) in the six biggest slaughterhouses, i.e. 75% of lots consist of less than 5 animals.

BT survey

With 60 samples per day and per slaughterhouse, the costs for slaughterhouse sampling break even with the costs for on-farm sampling after two weeks.

During this period, the system sensitivities for all initial herd level prevalences (0.01, 0.1 and 0.5) are below 75% at slaughterhouse level and show great variation (figure 4 C). As the on-farm sample size is calculated as a function of the desired sensitivity, the resulting sensitivity of almost 100% is not surprising (asterisk in figure 4 C). With the parameters chosen for the simulation model, the estimation of the herd-level prevalence is less cost-efficient with slaughterhouse sampling than with on-farm sampling.

For the slaughterhouse sample, the apparent herd-level prevalence is within the two standard deviations of the mean value for the lower initial herd-level prevalences (figure 4 A). The true herd-level prevalence at the slaughterhouse is lower than the initial herd-level prevalence in the population (figure 4 B). The MSE of the simulation (apparent prevalence vs. initial prevalence) suggests, that the slaughterhouse sample predicts the initial herd-level prevalence better than the on-farm sample for

low initial prevalences but that it produces poor estimates with high initial prevalences (figure 5).

At the animal-level, on-farm and slaughterhouse sampling perform well at the lower prevalences (i.e. 0.04 and 0.004 initial animal level prevalence). The slaughterhouse estimates plateau after less than two weeks of sampling (figure 4 D). At the animal level, the MSE shows that the slaughterhouse sample has the same precision as the on-farm sample for these lower prevalences (figure 5). With the break-even of the costs after two weeks, the slaughterhouse sample is less expensive than the on-farm sampling with a similar precision.

All cantons are represented in the slaughterhouse sample and the distribution of the farms to the cantons deviates only slightly from the distribution in the population with a maximum deviation of 15 % over all scenarios and runs (data not shown).

IBR/EBL surveillance

To demonstrate freedom from disease the sample sizes are large and on-farm sampling is costly. When sampling is restricted to animals over 2 years of age, the costs associated with slaughterhouse sampling are lower than the on-farm sampling scenario for all tested scenarios. The SSe of 99% can only be reached when also younger cattle are sampled, for the random and the dynamic sampling scenario, but the costs are twice as high as for the on-farm sampling. By sampling 200 animals per day for the entire year, the SSe reaches 90% for the scenarios ‘Dyn’ and ‘Lim’ and 95% for the scenario ‘Ran’. When sampling a maximum of seven animals per farm, the costs are lower for one year of sampling at the slaughterhouse, as compared to on-farm sampling (even when younger cattle are included (figure 6 and 7)).

The dynamic sampling scenario without limitation (Dyn) produces the largest deviation in the distribution of farms per canton of all scenarios. The other scenarios are able to reproduce the distribution of farms across cantons very well (figure 8),

Discussion

The results presented underline some inherent difficulties with slaughterhouse sampling caused by the fact that decisions to slaughter specific individuals are driven by economic or personal reasons of the farmer. In the Swiss system, this affects the herd-level sensitivity in the sample, as for most farms only a few (less than 3) animals are slaughtered over the course of one year. Thus a negative result for a farm is not informative about the true status of the farm. The authorities planning surveillance can therefore not decide in advance which farms, or how many animals per farm, should be in the sample to reach desired outcomes regarding system sensitivity or regarding the representativeness of a sample.

BT survey

For the simulated BT prevalence study, the underestimation of the herd-level prevalence in the slaughterhouse sample is attributable to the low herd-level sensitivity reached. For low initial herd-level prevalences the effect is masked by the imperfect test specificity. As the simulation allows the comparison of true and apparent prevalences, its outcome indicates that herd-level prevalences inferred from slaughterhouse surveys have to be interpreted with caution. The MSE illustrates how the estimates in the slaughterhouse sample even out with increasing sample size. For p^{bini}=0.5, the variation in the first days of sampling is higher than for the lower initial prevalence levels. The sampling period might be too short to cover the overall population. Especially dairy farms might slaughter cattle only on a few occasions throughout the year and are missed with a restricted sampling duration. Furthermore, with the implemented age restrictions, cows older than two years are excluded from the sample. Although we assumed they are protected by vaccination and therefore

not included in the marking, it affects the herd level sensitivity at slaughter. This leads to a possible bias in the slaughterhouse sample. The low herd level sensitivity and the potential underrepresentation of dairy farms in the sample lead to a lower prevalence in the slaughterhouse sample. For low initial herd-level prevalences the effect is masked by the imperfect test specificity. Also, the false positive animals are evenly distributed in the population and not restricted to farms with marked (i.e. true positive) animals. This can result in 'false positive farms' with no marked animal in the herd. This influences the apparent herd level prevalence more in scenarios with low initial between herd prevalence (i.e. p^{bini}=0.01 and 0.1).

The assumed within herd prevalence of 0.4 is based on values found in other northern European countries. We have no data on the within herd prevalences for BT in Switzerland as there were only very few farms infected and all data is from the very early stage of the epidemic in our country.

The results indicate that the slaughterhouse is not ideal for cross-sectional studies for prevalence estimation in Switzerland, and possibly also in other countries.

If inference on animal-level is the target, the slaughterhouse is more cost effective than on farm sampling, at least when the animal level prevalence in the population is lower than 10%. A random sample with one animal per farm in the sample is more easily achieved than with on-farm sampling. For the simulated BT prevalence survey, the same on-farm sample (ten animals per farm) was used for the herd-level and animal-level prevalence estimate. This is a compromise driven by economic considerations, as with one sample per farm, 3'500 farm visits would be necessary

which would result in very high costs. In the slaughterhouse, it is easier to select individuals from different farms of origin without increasing the cost of sampling.

IBR/EBL surveillance

To demonstrate freedom from disease, the low herd-level sensitivity increases the necessary duration of the surveillance programme. Of the tested sampling scenarios, a dynamic sample, favouring farms that already have animals in the sample after random sampling on day one but limiting the number of animals sampled per farm (Lim) is the most cost efficient overall. However, the SSe of 99% is still not reached. Such a scenario would require the implementation of a dynamic sampling algorithm with daily data driven optimization of the sample. By building an IT solution for the information transfer between the slaughterhouses and the veterinary service, one requirement for such a dynamic sample at the slaughterhouse is fulfilled. The implementation of a sampling algorithm could be envisioned as a future system enhancement.

Although current EBL/IBR surveillance concentrates on animals that are older than 24 months this is not a requirement of the bilateral treaty with the EU (Blickenstorfer et al., 2011). Non-dairy cattle older than 24 months are scarce in the slaughterhouse compared to dairy cattle in the same age category. The sample size (60 vs 200 per day and slaughterhouse) has little influence on cost and sensitivity of the programme for all scenarios when sampling animals older than 24 month, indicating that less than 200 cattle of this category arrive at slaughter per day in the study slaughterhouses. When the population of interest is expanded to non-dairy cattle older than 6 months the SSe increases, but obviously also the costs of the programme. If the costs are the limiting factor, focusing on the older animals and prolonging the sampling period would be the sampling scheme of choice.

Consequently, the population would be monitored all year round. Although this makes the start and end of a surveillance programme arbitrary, freedom of disease can be assured at lower cost than by on-farm sampling. Considering that the confidence gained in one year does contribute to the confidence for follow-up surveys (Hadorn et al., 2002), continuous surveillance at slaughterhouse will lead to desired confidence levels. If necessary, the sample could be complemented with on-farm blood samples to increase the SSe.

Limitations and applications

We simulated a cross-sectional prevalence study without introducing a transmission model for BT. Thus the model represents a situation after the BT epidemic is finished and the infection is no longer active, but where there are still seropositive animals in the population. We used a complete record of the Swiss cattle population for this study, as it provided an opportunity to investigate epidemiological concepts on farm- and animal level with real data. Our results could possibly be extended to other vector borne diseases, as long as there are no other possible transmission routes, or to assess vaccine coverage in a population. For other types of infectious diseases it would be necessary to include a state transition model for individual animals. This was beyond the scope of the present study.

The use of the real population data is a key feature of our work and allowed the simulation of scenarios close to reality. Using the controlled marking process prevalence values in the population (i.e. the initial prevalence) could be compared with the apparent and the true prevalence in the simulated sample. The disadvantage

of using the real movement data for 2012 is that the results cannot be implicitly generalized to other years.

The source population of 1.6 million cattle is large enough to generate some variation in the randomly tagged animals, but as the simulation uses real data the distribution of the animals across farms and the identity of the animals slaughtered are the same in every run. Therefore, the variation in the outcome variables is low. This is evident from most figures, showing very narrow confidence bands.

But as long as slaughter customs and numbers stay more or less constant, the results of the simulation should be applicable to the Swiss cattle population in the future. A study on the dynamics of the Swiss cattle population showed that the population was in equilibrium over the years 2009-2011 (Schärrer et al., 2014). There is no evidence or predictions of drastic changes in the population in recent years or in the near future.

The presented analysis is only considering the costs for surveillance. The added 'soft' benefits (Howe et al., 2012) if sampling is shifted from on-farm to the slaughterhouse for the non-dairy population are not considered in the model. The operational hazards for official veterinarians and farmers would decrease and the farmers would gain time as they would not have to assist with sampling anymore by gathering and restraining their cattle. Additionally, the non-invasive, post mortem sampling can be considered more welfare-friendly.

Over all, the slaughterhouse as a centralized infrastructure, where a great number of specimens arrive from all geographical regions of Switzerland, proves to be a valid alternative to on-farm sampling, given the sampling frame is carefully chosen. The comparison of different sampling scenarios showed that most benefit from shifting the

sampling to the slaughterhouse is gained when a dynamic sampling scheme is applied or the target is defined on animal level. With the implementation of an IT-system allowing for dynamic or targeted sampling, the slaughterhouse can be used for the proof of absence from disease, or for ad-hoc surveillance programmes on animal level, for example for the early detection of emerging disease. This could be of special interest if, for example, a disease is spreading in a neighbouring country towards the Swiss border, and the most likely geographical region of an introduction can be defined and used as a risk factor for a targeted survey.

Conclusion

With the introduction of an IT-solution allowing the allocation of individual cattle or farms to a sample and to introduce dynamic sampling scenarios, the Swiss veterinary service will gain a powerful and flexible tool for surveillance. If the solution is a complete shift to slaughterhouse sampling, or if a combination of on-farm and slaughterhouse sampling is more adequate, has to be decided according to the disease and surveillance requirements under consideration.

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References

Anonymous, 2002. Agreement between the European Community and the Swiss Confederation on trade in agricultural products.

Anonymous, 2014. Bericht zur Überwachung von Tierseuchen und Zoonosen.

Blickenstorfer, S., Schwermer, H., Engels, M., Reist, M., Doherr, M.G., Hadorn, D.C., 2011. Using scenario tree modelling for targeted herd sampling to substantiate freedom from disease. BMC Vet. Res. 7, 49. doi:10.1186/1746-6148-7-49

Buchi, M., Abril, C., Vögtlin, A., Schwermer, H., 2014. Prevalence of antibodies against bluetongue virus serotype 8 in bulk-tank milk samples from dairy cattle herds located in risk a reas for bl uetongue virus transmission after a vaccination programme in Switzerland. Berl. Munch. Tierarztl. Wochenschr. 4, 158–162. doi:10.2376/0005-9366-127-158

Cameron, A., Baldock, F., 1998. A new probability formula for surveys to substantiate freedom from disease. Prev. Vet. Med. 34, 1–17. doi:10.1016/S0167-5877(97)00081-0

Christensen, J., Gardner, I.A., 2000. Herd-level interpretation of test results for epidemiologic studies of animal diseases. Prev. Vet. Med. 45, 83–106. doi:10.1016/S0167-5877(00)00118-5

Durand, B., Zanella, G., Biteau-Coroller, F., Locatelli, C., Baurier, F., Simon, C., Le Dréan, E., Delaval, J., Prengère, E., Beauté, V., Guis, H., 2010. Anatomy of bluetongue virus serotype 8 epizootic wave, France, 2007-2008. Emerg. Infect. Dis. 16, 1861–8. doi:10.3201/eid1612.100412

Hadorn, D., Rüfenacht, J., Hauser, R., Stärk, K., 2002. Risk-based design of repeated surveys for the documentation of freedom from non-highly contagious diseases. Prev. Vet. Med. 56, 179–192.

Howe, K.S., Häsler, B., Stärk, K., 2012. Economic principles for resource allocation decisions at national level to mitigate the effects of disease in farm animal populations. Epidemiol. Infect. 141, 91–101. doi:10.1017/S095026881200060X

Lynch, J., Silva, P., 2013. Integrating animal health and food safety surveillance data from slaughterhouse control. Rev. Sci. Tech. 32, 409–416.

Martin, S., Shoukri, M., Thorburn, M., 1992. Evaluating the health status of herds based on tests applied to individuals. Prev. Vet. Med. 14, 33–43. doi:10.1016/0167-5877(92)90082-Q

Niedbalski, W., 2011. Evaluation of commercial ELISA kits for the detection of antibodies against bluetongue virus. Pol. J. Vet. Sci. 14, 615–619.

R Development Core Team, 2008. R: A language and environment for statistical computing.

Reber, A., Reist, M., Schwermer, H., 2012. Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhino-tracheitis and bovine enzootic leucosis in Switzerland. SAT 154, 189–197. doi:10.1024/0036-7281/

Reist, M., Jemmi, T., Stärk, K., 2012. Policy-driven development of cost-effective, risk-based surveillance strategies. Prev. Vet. Med. 105, 176–84. doi:10.1016/j.prevetmed.2011.12.014

Schärrer, S., Presi, P., Hattendorf, J., Chitnis, N., Reist, M., Zinsstag, J., 2014. Demographic model of the Swiss cattle population for the years 2009-2011 stratified by gender, age and production type. PLoS One 9, e109329. doi:10.1371/journal.pone.0109329

Schärrer, S., Reist, M., Lindberg, A., Guichard, M., Schulz, C., Fahrion, A., 2013. Feasibility of slaughterhouse sampling for surveillance of beef cattle in Switzerland. FLWI 6.

Schwermer, H., Chaignat, V., Hadorn, D., Schärrer, S., Schaffner, F., Thür, B., Breidenbach, E., 2008. Das Überwachungsprogramm der Blauzungenkrankheit in der Schweiz. Schweiz. Arch. Tierheilkd. 3, 129–132.

Schwermer, H., Reding, I., Hadorn, D., 2009. Risk-based sample size calculation for consecutive surveys to document freedom from animal diseases. Prev. Vet. Med. 92, 366–372. doi:10.1016/j.prevetmed.2009.08.021

Sergeant, E., 2012. Important formulae for surveillance [WWW Document]. URL http://epitools.ausvet.com.au/docs/Important-formulae-for-surveillance.pdf

Van Schaik, B., H, van L., Elbers, A., Vellema, P., 2008. Seroprevalence of bluetongue serotype 8 in cattle in the Netherlands in spring 2007, and its consequences. Vet. Rec. 163, 441–444.

Willgert, K., Schroedle, B., Schwermer, H., 2011. Spatial analysis of Bluetongue cases and vaccination of Swiss cattle in 2008 and 2009. Geospat. Health 5, 227–237.

Table 1: Possible outcome for individual cattle after the marking process for the BT survey simulation. FN: false negative; FP: false positive

marked	FN	FP	Sample*	Outcome
1	1	-	1	In the sample, 'positive', not detected
1	0	-	1	In the sample, 'positive', detected
1	1	-	0	Not in sample, 'positive' in prevalence estimate in the living population
1	0	-	0	
0	-	1	1	In the sample, 'negative', detected as positive
0	-	0	1	In the sample, 'negative', not detected
0	-	1	0	Not in sample, 'negative' in prevalence estimate in the living population
0	-	0	0	

*on-farm or slaughterhouse-sample; an animal can possibly end up in both samples

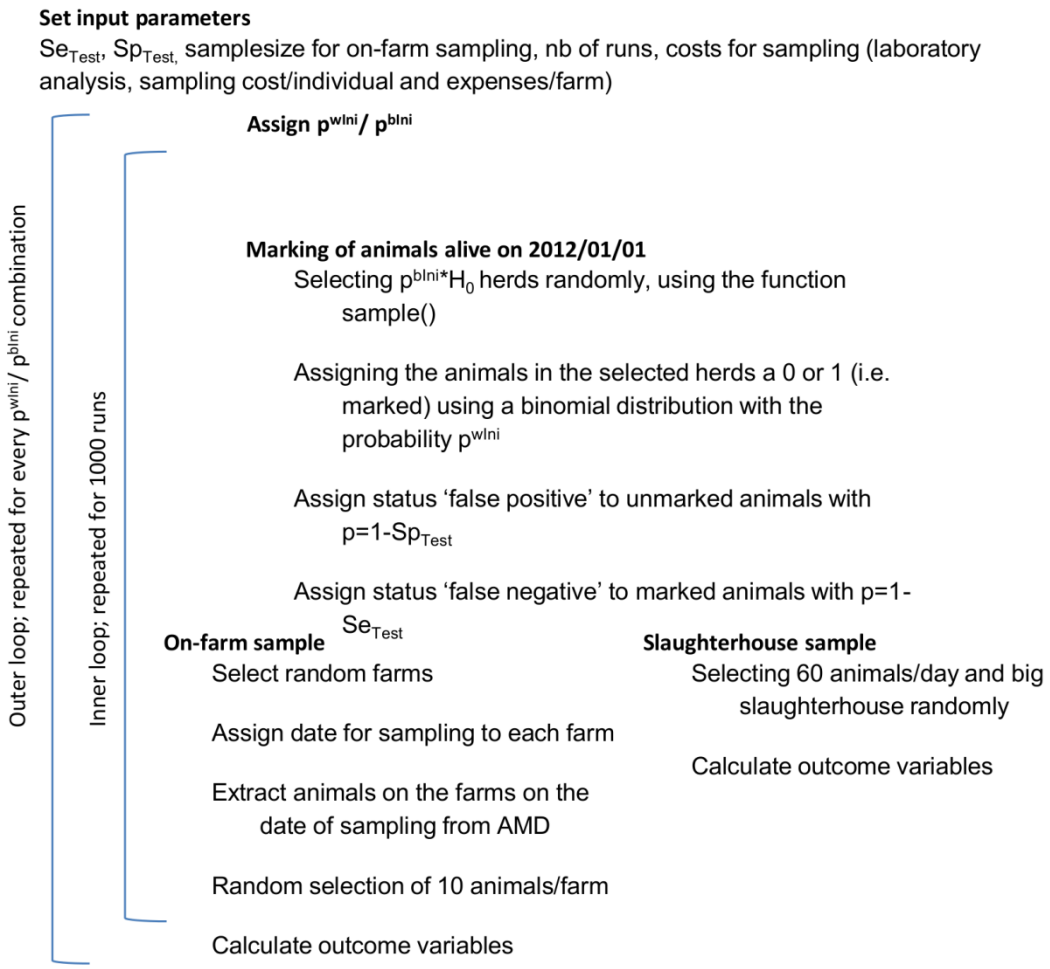

Figure 1: Schematic representation of the bluetongue prevalence simulation model.

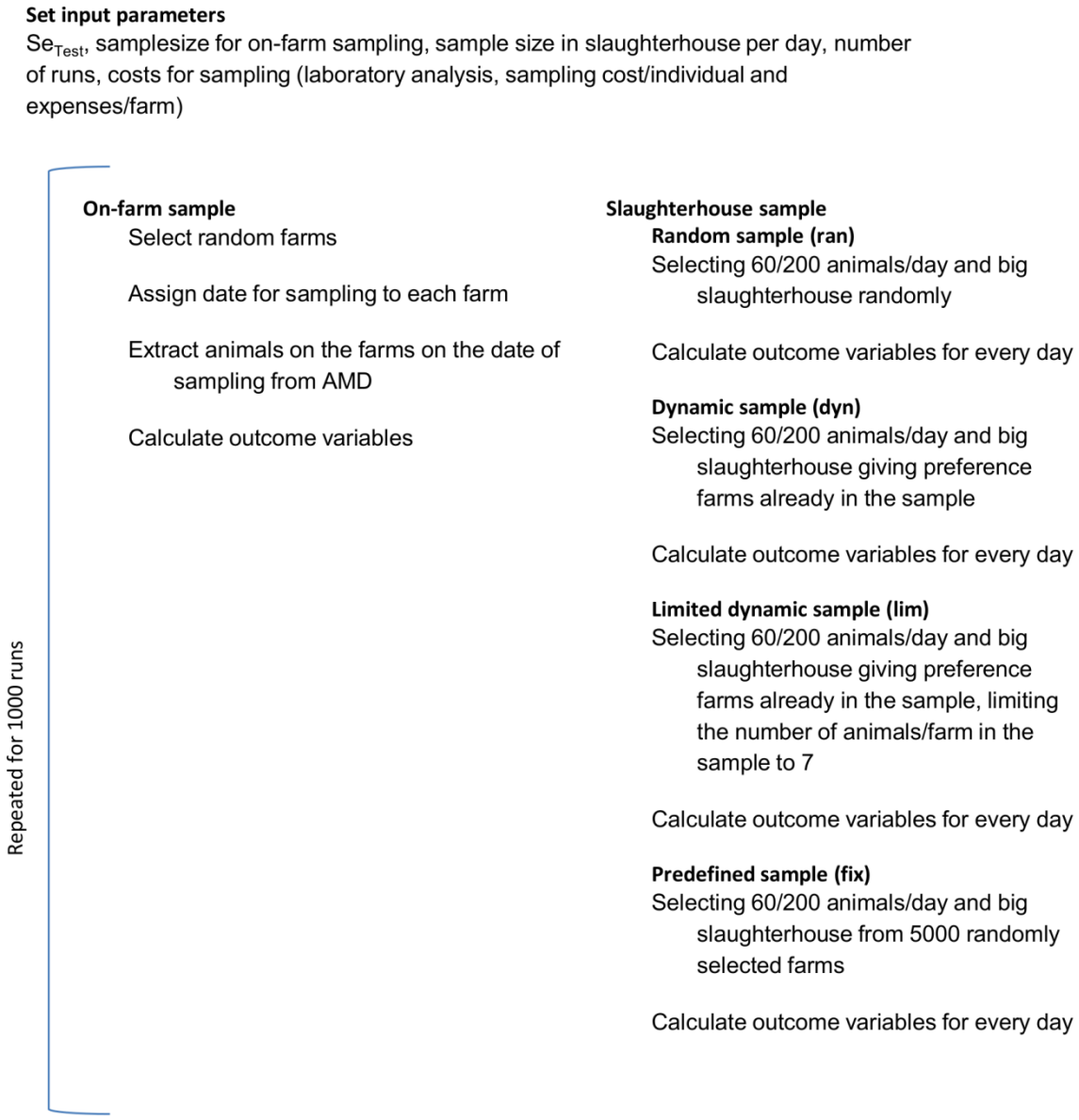

Figure 2: Schematic representation of the simulation model for freedom from disease.

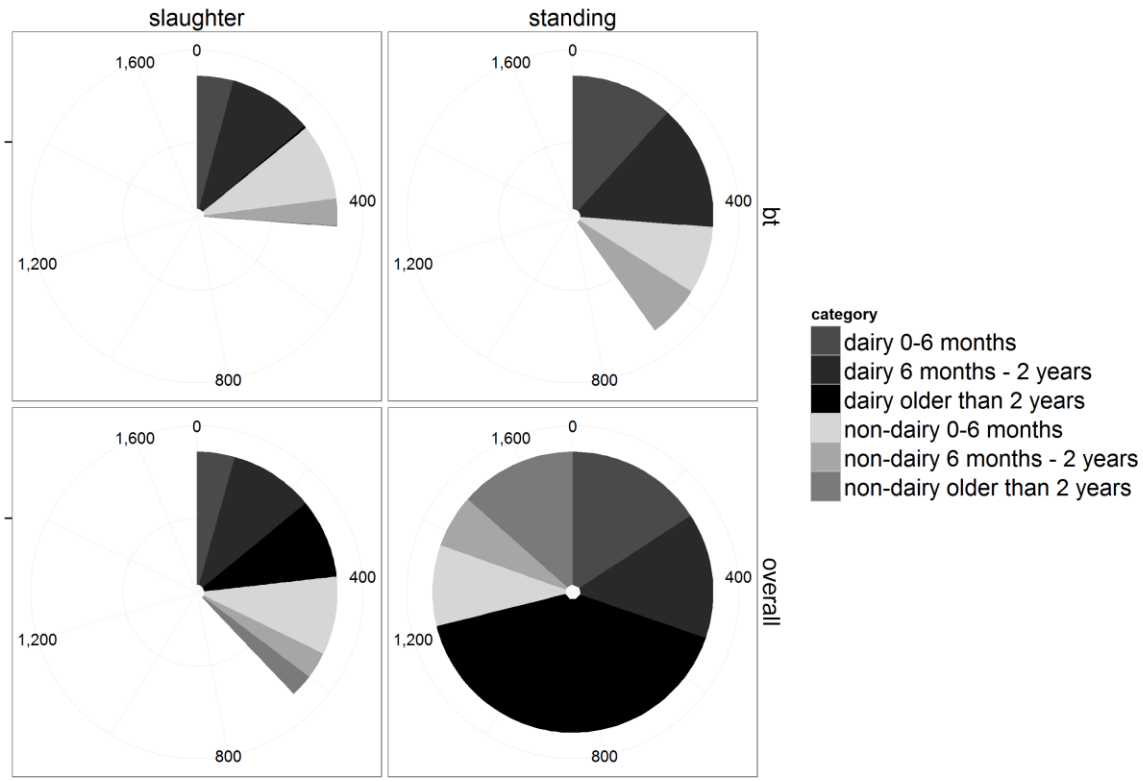

Figure 3: The count of bovines in thousands in the standing population on the 1st of January 2012 and in the slaughter population (SH) in 2012. In the upper row the sub-population containing only animals borne after the 2010/06/30 is denoted ‘bt’ and refers to animals eligible for the simulated bluetongue survey.

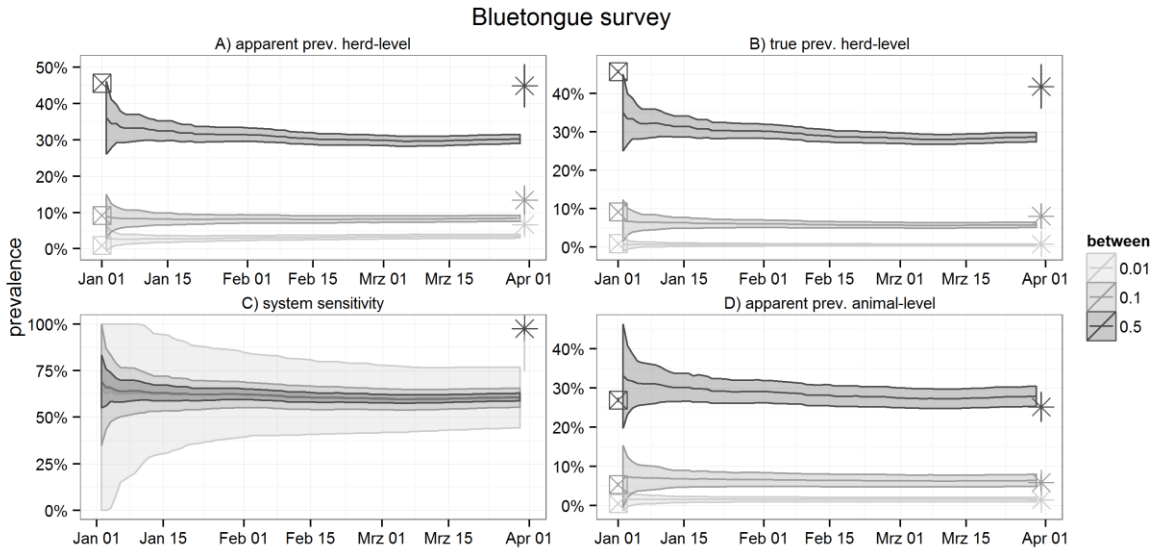

Figure 4: Apparent herd-level prevalence (A) true herd-level prevalence (B), system sensitivity (C) and animal-level prevalence (D) in the slaughterhouse sample for the BT simulation. Crossed box: prevalence of marked animals in the population at the herd- or animal-level. Asterisk: apparent and true herd-level prevalence, animal-level prevalence and system sensitivity in the on-farm sample with error bars of two standard deviations. Solid lines: mean apparent and true herd-level prevalence and system sensitivity in the slaughterhouse sample; grey areas: corresponding interval of two standard deviations. From lighter to darker grey: initial between herd prevalence of 0.01; initial between herd prevalence of 0.1; initial between herd prevalence of 0.5.

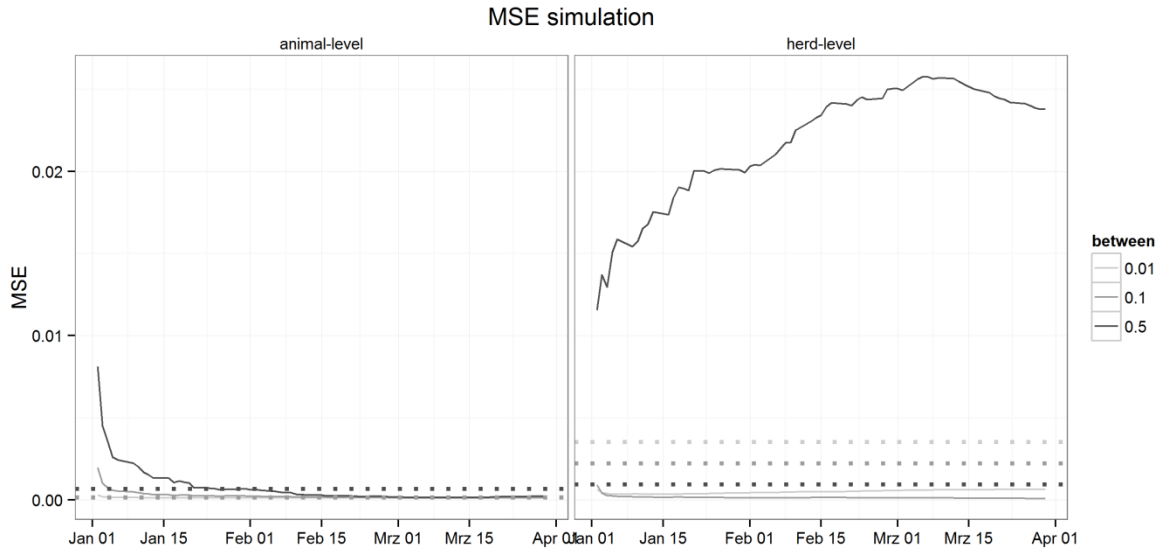

Figure 5: Mean squared error (MSE) for the apparent herd-level and animal-level prevalence estimates in the slaughterhouse sample (solid lines) and the on-farm sample (dotted lines) for the bluetongue survey. The lower the mean squared error, the more accurate is the overall performance of the sampling location. From lighter to darker grey: initial between herd prevalence of 0.01; initial between herd prevalence of 0.1; initial between herd prevalence of 0.5.

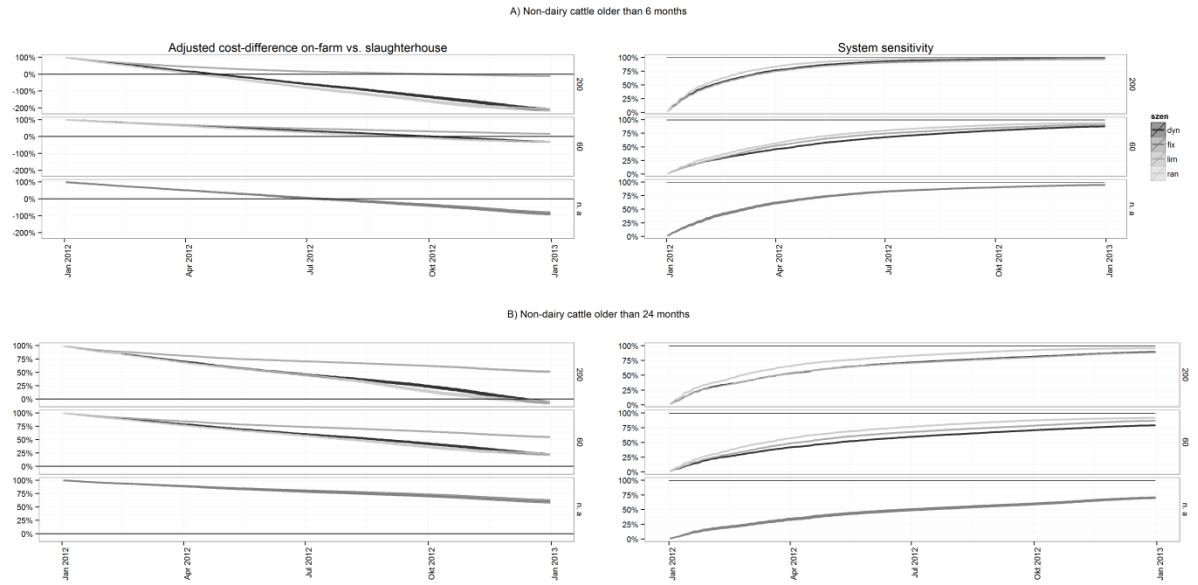

Figure 6: Adjusted cost difference for four herd-level sampling scenarios. A) cattle older than 6 month; B) cattle older than 24 month. Cost difference is calculated as the difference of the total cost of on-farm sampling and the summarized cost for the slaughterhouse sample for every day divided by the total cost of on-farm sampling (mean values from the simulation) and system sensitivity. Coloured areas indicate the interval of two standard deviations. The horizontal black line indicates the break-even point. Sampling scenarios from lighter to darker grey: dynamic sample favouring farms that already have cattle in the sample (dyn); sample exclusively from 5000 randomly selected farms (fix); dynamic sample but limiting the number of animals per farm in a sample to 7 (lim); random sample (ran). The right-side axis indicates the number of samples taken per day (60 or 200). For the scenario fix, the sample size depends on the number of animals from the given farms that arrive at slaughter (not applicable, n.a.)

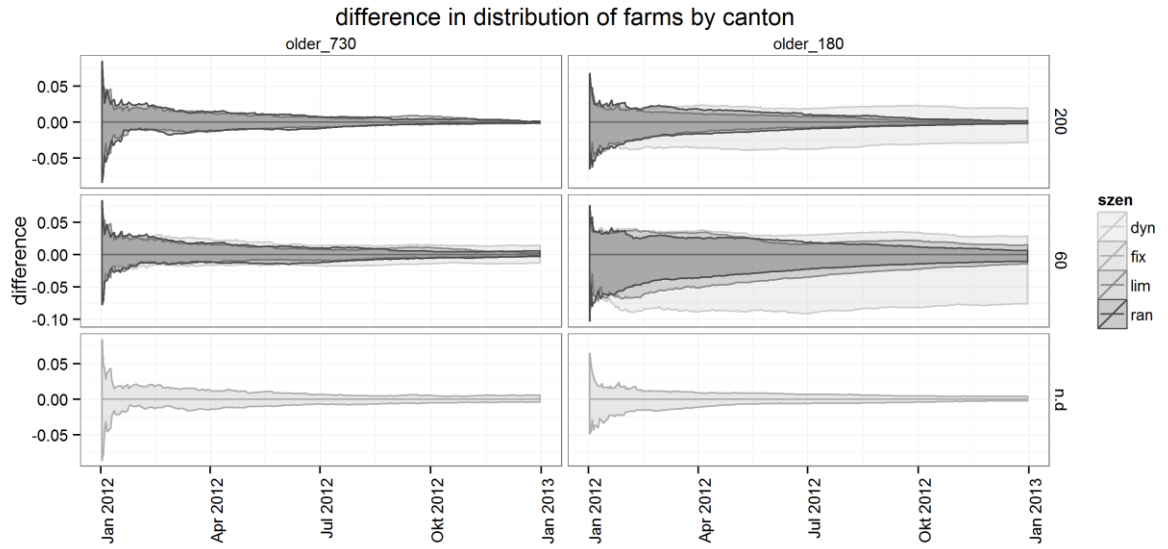

Figure 7: Mean difference of the proportion of farms from a canton in the slaughterhouse sample compared to the overall population. Grey areas show the minimum and maximum values of the difference over all runs. Sampling scenarios from lighter to darker grey: dynamic sample favouring farms that already have cattle in the sample (dyn); sample exclusively from 5000 randomly selected farms (fix); dynamic sample but limiting the number of animals per farm in a sample to 7 (lim); random sample (ran). The right-side axis indicates the number of samples taken per day (60 or 200). For the scenario fix, the sample size depends on the number of animals from the given farms that arrive at slaughter (not applicable, n.a.)

11 General discussion and conclusions

11.1 Surveillance at the slaughterhouse

11.1.1 Dairy and non-dairy cattle population in Switzerland

The main motivation of the PhD Project was to analyse the use of slaughterhouses as sample source for routine surveillance in Switzerland and in this way to explore further possibilities to render MOSS more cost efficient. For dairy herds there exists already a cost-efficient way for sampling by making use of bulk tank milk testing, as a centralized collection system for milk samples is in place for the mandatory, biweekly quality testing. With its introduction in 2010 (Reber et al. 2012; Reist et al. 2012), the national surveillance programmes were inevitably divided in two components according to production type. However, for diseases that cannot be tested in bulk tank milk and – more importantly – always for the non-dairy sector, on-farm sampling is still the method of choice. Therefore, in a future implementation of sampling at the slaughterhouse for surveillance programmes, mainly the non-dairy sector has to be covered. The introduced population model was therefore stratified by production type (dairy or non-dairy), age and sex (male and female calves: 0-1 year, heifers and young bulls: 1-2 years, cows and bulls: older than 2 years). With the chosen stratification the structural dynamic in age and sex classes conditioned by a farming system focused on dairy production was well captured and the expected differences in management practices of dairy and non-dairy farmers are reflected.

The value of females in the dairy sector is reflected by high survival rates throughout the age classes. Male calves on the other hand have higher mortality rates than female calves and are irrelevant in numbers after one year of age. Other authors found similar differences in mortality rates for male and female calves (Bleul 2011; Perrin et al. 2011) and this difference can at least partially be explained by the lesser

economic value of male animals for dairy farmers. They are more willing to invest in females with good linage in terms of veterinary costs or time of caretaking than in males that are sold for fattening or slaughter anyways. In the non-dairy sector, also very few bulls survive after the age of two, but more male calves than female calves are found in the non-dairy sector. At slaughter, the proportions in the strata differ. Young males are the most valued commodity for meat production and are therefore slaughtered in high numbers. Heifers are rarely slaughtered in both production sectors. Dairy cows older than two years make the biggest share at slaughter because of their predominance in the living population (i.e. a third of all living animals are in this stratum). Knowing this, samples taken at the slaughterhouse can be corrected for age and sex to estimate the prevalence in the living population if necessary, for example if differences in prevalence are expected in different age categories.

The number of cattle in each stratum was constant during the study years, suggesting that farmers keep their herds stable and the market is fully exploited.

The fitted rates derived from the model allow the simulation of the population for future models. As example, we created a matrix model of the Swiss cattle population defining the eigenvalue of the equilibrium population (Chapter 14 Appendix 2). Sampling schemes can be planned as the numbers of animals to be expected at the slaughterhouse is known (Chapter 6 & 10).

In spite of the differences in management practices and the necessity to consider the dairy and non-dairy sector as different sub-populations for surveillance in consequence of the implemented bulk milk testing, there is no epidemiological or geographical division between the sectors. In fact, the non-dairy sector is restocked to a large degree with dairy calves. In the network analysis where age and sex of the

moved animals were not considered, the only difference according to the production type of a farm was found in the number of direct outgoing contacts (out-degree). The higher values found for dairy farms compared to non-dairy farms are attributable to the calves sold for fattening. Otherwise the results indicate that the production type of a farm has little influence on its position in the network. The small size of the country and the high density of holdings lead to an interconnected network. Cattle traders will buy calves from the dairy sector and sell them to fattening plants but will also trade animals within the sectors, creating criss-crossed movement patterns.

The production type of the farms is declared by the farmer. There are three possible entries in the AMD; ‘dairy cows’, ‘other cows’ and ‘mixed use’. For the project, ‘other cows’ and ‘mixed use’ was summarized as ‘non-dairy’, because the number of farms in the ‘dairy’ category concurred with the number of farms that deliver consumer milk and had milk quality tests in 2011. The two data systems (AMD and milk quality database) have different identifiers for the farms and are not matchable. The results of the population model for the proportion and differences in the strata for the production types are plausible. The AMD is the information source used for planning and conducting surveillance. For future programmes comprising slaughterhouse sampling it should be validated which farms are covered by bulk milk testing.

11.1.2 Practical feasibility

In the current system, the samples for the surveillance of non-dairy cattle are gained on-farm. The procedure is organized by the cantonal veterinary authorities after the sampling scheme and stratification by canton is fixed by the FSVO. With the aspired shift of on-farm sampling to slaughterhouse sampling, organisational changes are unavoidable. While it was assumed that overall the sampling at the slaughterhouse

would cost less than the on-farm sampling, it was also evident that for the slaughterhouses costs and workload would increase.

If the slaughterhouses are to be used for the yearly routine surveillance programmes, the sampling must be integrated in the routine of the slaughterhouse and meat inspection personnel. It was an important part of the project to integrate the experience and opinions of the veterinarians working at the slaughterhouse for meat inspection as basis for the decision-makers at the federal level. Focusing on the six biggest slaughterhouses in Switzerland, the collaboration was sought at an early stage. The main goal of the conducted feasibility study was to assess the best method and sampling material at the slaughterhouse and gain an idea about the feasibility and the generated additional costs for sampling.

It is noteworthy, that all of the contacted meat inspectors were open for collaboration and participated actively in the study. The experience gained in the accompanied slaughterhouse visits are a vital part of the whole PhD project. It was also striking, that even though the workload and administrative effort would increase, there was a shared agreement and understanding, that using the slaughterhouses more efficiently for national surveillance is a logical and necessary step for the Swiss veterinary service. They also agreed that the sampling must be conducted by official veterinarians and not by slaughterhouse employees.

The feasibility study showed that without a system to assign bovines electronically for sampling and to detect assigned animals in the slaughter chain at meat inspection, no selection of whatsoever criteria for risk based sampling is reliable at slaughter (chapter 8). As at least the differentiation of dairy and non-dairy cattle was a pre-condition for developing sampling at slaughterhouse as strategy for routine surveillance, this was an important outcome.

The FSVO is building currently an IT system designed to assign farms or individual cattle to a specific surveillance programme and transmit this information to the slaughterhouse, where systems to detect animals assigned for sampling in due time and at the right place will be installed.

As for the sampling matrices, blood samples are the most likely to serve the purpose for routine surveillance. If the daily sample load is not too high, the blood can be gained from the heart ventricles during meat inspection. Meat juice would be a valid alternative and could facilitate the logistics for sampling at the slaughterhouse considerably as it could be gained as long as the carcass is accessible. It therefore offers a much longer time window than any other sampling material. The diagnostics for the commonly monitored bovine diseases should be developed and validated for meat juice to offer further flexibility for surveillance programmes at slaughterhouse level.

11.1.3 Customs and practices within the Swiss cattle industry relevant for surveillance at the slaughterhouse

Slaughterhouse surveillance is not new and implemented in many countries (Lynch & Silva 2013). Switzerland conducts surveillance programmes of pigs and poultry at slaughterhouse level (Anonymous 2014). In contrast to pigs and poultry, in the Swiss cattle industry all in/all out production cycles are limited to some fattening plants and cattle are rarely slaughtered in batches. Bovines from one farm arrive in a seemingly random pattern at slaughter. Most farmers deliver cattle to several slaughterhouses in the course of one year and slaughter one animal at the time. Small cattle trading operations collect bovines destined to slaughter from farms and deliver them to the slaughterhouse at the same day. The big slaughterhouses have contracts with different retailers and according to the preference of those, price levels might differ for specific criteria. To optimize the price at slaughter, animals from one farm might

end up in different slaughterhouses on the same day. Adult cows are only slaughtered if they are not productive anymore. The farmers presumably decide to send those animals to slaughter based on current events like choosing between treatments or slaughter in case of a health event, the necessity to make room for new breeding stock or bringing them to alpine pastures or not. These individual based decisions ad to the random pattern observed at the slaughterhouse (Chapter 8, 10 & 15, Appendix 3).

As already mentioned, the confidence to detect diseased herds in the population depends amongst other on the number of animals sampled per herd (Martin et al. 1992; Cameron & Baldock 1998; Cannon 2001). As a consequence, reliable herd-level conclusions on the health status of the cattle population at the slaughterhouse are difficult to obtain.

11.1.4 Potential bias at the slaughterhouse

It is commonly assumed that sampling at the slaughterhouse is biased for different reasons, although there is barely literature published on the subject. The most evident reason is the different age and sex structure of the slaughtered compared to the living cattle population. However this is only relevant for monitoring and surveillance systems if the prevalence of the disease or hazard under investigation differs in various age or sex classes. If such differences are known, the surveillance programme can be planned accordingly by stratifying the sample or by limiting the slaughterhouse sample population to the living segment population of interest. This is done for example for the proof of absence from EBL in Switzerland (Blickenstorfer et al. 2011) for which only cattle older than two years are sampled. If the differences are only suspected, the obtained prevalence estimates in the sample can be corrected for. By deducing the multipliers from the outcomes of the population model (chapter

6), the proportion of the strata in the slaughterhouse sample can be adjusted to the living population.

As currently the routine surveillance programmes at the slaughterhouse will be limited to the big slaughterhouses for practical reasons, the geographical representativeness of the sample needed to be assessed. These slaughterhouses all operate from the Swiss plateau (see chapter 13, Appendix 1). The Alps act as natural barrier to the south and it was assumed that southern Switzerland (cantons Valais and Ticino) would be underrepresented in the sample. The geographical representativeness was inquired with two different methods; on cantonal level using the model described in chapter 10 and on zip code level using network analysis techniques (Chapter 14, Appendix 2). Considering the monthly pictures from the slaughter basin as potential coverage in a sample, it is visible that not all zip code areas are covered every month and that missing data points are mostly located in southern Switzerland. On cantonal level and with a simulated sampling scheme, the geographical coverage is comprehensive with every canton sampled. If the coverage of southern Switzerland must be warranted however, the local slaughterhouses should be included in the planning of the surveillance activity to take some additional samples. Such collaboration was implemented for the prevalence study of ESBL producing *Enterobacteriaceae* (Chapter 9).

Of the potential biases discussed the most worrisome is the difference in animals sent to slaughter in small slaughterhouses compared to the animals sent to the big, industrial ones. Farmers sending animals to the larger slaughterhouses, often as part of a quality label programme or a supply agreement, also tend to apply higher level of quality self-control as a result of pressure from private retailers. The ante-mortem inspections are more rigorous and emergency slaughter are not offered as service but merely conducted if animals arrive with injuries from the transport. It is suspected,

that some of the small slaughterhouses (there are over 500 slaughterhouses registered in Switzerland) are better adapted to process also animals in a lower general status. As a consequence, such animals are likely to end up in a small slaughterhouse.

Especially for the early detection of emerging diseases and for using welfare indicators at slaughterhouse level for benchmarking purposes, the small slaughterhouse should be integrated in the surveillance system.

11.1.5 Sampling schemes and cost comparison to on-farm sampling

It is evident, that an IT-infrastructure must be in place to fully exploit the possibilities of slaughterhouse sampling. Once such a system is in place, the slaughterhouse can be used in a flexible way for surveillance programmes. So far, only the recognition of previously assigned individual animals or farms at the slaughterhouse is implemented in the IT system in development. The chosen individuals or farms are fed into the system as static lists. Although this allows selecting farms or animals from a production system or implementing some selection criteria of individuals like age or sex, the influence on the sensitivity of the surveillance programme is little. To improve the system sensitivity, i.e. to have more control over the number of farms and/or the number of animals per farm in the sample, a dynamic allocation of cattle to the sample must be possible. The simulation of the costs of sampling and the reached system sensitivities (chapter 10) showed that a dynamic sampling scheme would achieve the same sensitivity level as a random sample but with lower costs.

In the cost comparison, hidden costs such as time expenses of the farmer to corral and fixate the animals or risk of injury for the veterinarian and the farmers were not considered. Considering these hidden costs would have made slaughterhouse sampling even more interesting.

11.1.6 Transdisciplinarity

The present study is in large parts based on the AMD, using different approaches to analyse the data. This theoretical approach allowed us to tackle different problems on national level. The data collected in the AMD – containing information about every bovine in the Swiss cattle industry – made a profound analysis and description of the overall population possible and additionally gave some insight how the management practices are reflected in the data. However, this purely data driven view of real-world processes bears the risk of missing important information. The feasibility study and discussions with experts in the field, university and government were an important part of the project for validating assumptions and gaining confidence in the produced modelling outcome.

When the shift of sampling from on-farm to the slaughterhouse is implemented, this transdiciplinary approach needs to be pursued (Schelling & Zinsstag n.d.). The stakeholders at the slaughterhouse (meat inspectors, owners and providers of IT-solutions) are gaining the samples and delivering information referring to them. They have to deal with practicalities and can though provide essential insight in practical issues. They also will notice changes in the slaughter population before the central authorities. Meat inspectors are trained to ensure food safety and hygiene and have a professional understanding of public health. The close collaboration of the national veterinary services and the meat inspectors in the big slaughterhouses is a chance to enhance the understanding of health issues of public concern of both parties and to increase the sensibility for arising future challenges like emerging diseases or changes in the farming systems with implications for control strategies.

11.2 Deriving risk factors from the AMD

11.2.1 Risk factor analysis

Other studies used national data sources to derive risk factors in cattle farms in Switzerland. Presi et al. (2008) used the number of incoming movements and herd size to derive the risk of tetracycline residues found in slaughter calves. Blickenstorfer et al. (2011) used data from the AMD to derive high risk strata for the targeted surveillance of IBR and EBL (above average incoming movements, summer pasturing and import of cattle). Both studies used data summarized over one year.

For the risk factor analysis of extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae,* an individual-based approach was used and the live-history of the animals in the study was taken into account. The farm on which the sampled animal spent the most time during its lifetime was used as reference. All risk factors were calculated for the time-period the animal spent on this farm and the estimates are therefore more specific to the sample population. The advantages of performing risk factor analysis with such data are pointed out in chapter 9. Full access to the AMD data is a valuable asset for the Swiss veterinary service, offering many possibilities to refine and improve knowledge on the animal health status and associated risks with moderate time and personal effort.

The confirmation of established reservoir of ESBL producers in, especially in dairy farms, is worrisome. The gained intelligence could improve management strategies in animal holdings towards a reduction of ESBL prevalence in cattle. A more prudent use of antibiotics, especially of 3rd and 4th generation cephalosporins, restrictions in feeding milk of treated cows to calves and changes in management practices towards closed herd policies could represent modes of action to achieve this.

11.2.2 The use of network parameters for targeted surveillance

Although cattle movements in terms of animals arriving on a farm were used as risk factor for disease introduction (Blickenstorfer et al. 2011), residuals (Presi et al. 2008) or ESBL producing *Enterobacteriaceae* (Chapter 9) so far neither the movement network itself nor the position of farms in it were considered in Switzerland to your knowledge.

Several countries used similar data for the analysis of cattle movements and the use of network parameters to inform risk based surveillance was suggested. The number of direct contacts of farms can be used to identify premises with an important role in the contact network and hence a priority for targeted surveillance (Nöremark et al. 2011; Frössling et al. 2012; Bigras-poulin et al. 2006; Mweu et al. 2013).

The chosen approach for the network analysis of the cattle movements in Switzerland in 2012 resulted in descriptive parameters for every cattle farm. The Swiss cattle movement network is scale free like it was described for other animal movement networks (Woolhouse et al. 2005; Nöremark et al. 2011; Kiss et al. 2006), meaning that very few farms have a lot of direct contacts while most farms have few direct contacts. This would make the in-degree an adequate criterion for risk based surveillance, targeting the farms at the high end of the power distribution.

In the case of Switzerland however, chronological sequences of contacts (i.e. ICC, see chapter 7) grow very large in the course of one year, indicating that the farms can be reached from most other holdings following the contact pattern in time and space. When assuming that farms with high ID and high ICC bear the most information about the population health status, the high chain measures in Switzerland make it difficult to set a cut-off for risk strata. This is emphasized by the facts that ID and ICC are only moderately correlated and that a farm with few direct ingoing contacts can have high ingoing chain values. Based on the present study, the

parameters resulting from the yearly network analysis are therefore not very promising factors to inform risk based surveillance systems on farm level.

Another factor to consider is the time depended topology of animal movement networks (Konschake et al. 2013; Mweu et al. 2013). The composition of farms within the network changes over time, as the farms do not have animal movements on a daily basis. Depending on the chosen time scale, a farm might be in the network or not, might have in and outgoing contacts or contacts in only one direction and so forth.

In case of a disease event, prioritizing farms with high outgoing contacts for control is sensible, but the data to assess the contacts must be up-to-date and the time window for the analysis must be relevant for the disease situation. In this context one shall not forget that farmers have a term of three working days to notify movements and that a delay in the dataset is therefore inevitable.

11.2.3 The risk associated to the geographical location in the network

Methods for network analysis were developed in the context of social interaction (Martinez-López et al. 2009). In the case of animal movement networks, abstract links between nodes become very real because animals are transported in vehicles on roads. While in theory edges can be weighted by the number of animals moved between the same nodes and therefore classified, in reality these links are not linear distances. The most frequented road segments may put nearby farms at a higher risk of infection, even if they have only few contacts in the network to other farms.

As far as we know, we are the first to map animal movements in Switzerland on the road net, using the fastest route from the holding of origin of a movement to the holding of destination (Chapter 7). Weighting road segments according the number of animal transports passing by, a risk map for the frequency of animal traffic could be

created. Assuming that passing-by animal traffic could be source of infection through contaminated dust, dirt or faecal particles and wheels, airborne diseases or insects traveling with the animals (vectors like biting midges or mechanical transmission trough flies) (Rivas et al. 2003), the farms close to highly trafficked road segments are at risk.

The resulting monthly maps indicated that farms on the Swiss plateau alongside the highway between Bern and Olten are at particular high risk for disease spread through animal transports.

11.3 Conclusion

Sampling at slaughterhouse level is a valid alternative to on-farm blood sampling if some conditions are met. If the outcome of the survey is on animal level, the slaughterhouses provide a flexible sampling location where hundreds of specimens are accessible daily. For herd-level outcomes, the programme must be planed carefully and a longer sampling period is necessary to reach reasonable system sensitivities.

In both cases, the implementation of an IT-system to allocate individual cattle to the sample is necessary, if the potential of slaughterhouse sampling is to be exploited. If the system allows also for dynamic sampling allocation, the sampling at the slaughterhouse-level becomes also at herd-level more cost-efficient than on-farm sampling covering the same population.

The low herd-level sensitivity in slaughterhouse samples is critical. Even with a year round sampling scheme the system sensitivity may not reach desired levels if the sampling population is restricted (production type, age or other criteria). However, to assure that the requirements of the bilateral treaty with the EU or other trading partners are met, the sample could be planned as combination of on-farm and slaughterhouse sampling or – as the dairy and non-dairy populations are epidemiologically linked – as combination of slaughterhouse and bulk milk sampling.

From a practical perspective, the sampling at the slaughterhouse is not a problem. The official veterinarians conducting the meat inspection are conscious of their role in veterinary public health. They are willing to implement some organisational changes to integrate routine sampling in their every-day work load. However, the necessary personal resources need to be considered. So far, taking blood from the heart ventricle seems to be the most reasonable sampling strategy for the currently

implemented surveillance programmes in cattle. However, the further development of meat juice diagnostic would open up some leeway for the organisation of the sampling.

The chosen approaches to describe the Swiss cattle population complement each other; while the introduced demographic model captures the structural dynamic in age and sex classes created by a farming system focused on dairy production, the network analysis revealed seasonal patterns and characteristics in the movement network that are driven by tradition and individual decisions of the animal owners. The conducted network analysis gave insight to some characteristics of the Swiss cattle industry and creates a basis for further exploration of the movement network.

By mapping the movements on the Swiss road system, the risk of the farms in relation to their vicinity of the most trafficked roads could be qualitatively assessed. This information could be used for risk based surveillance or as starting point for disease investigation in case of an outbreak.

The network measures used to describe the Swiss cattle trade network gave an intuitive access to the challenges for disease mitigation in Switzerland. However, the temporal dynamics of the network makes the use of the measures complicated. Although the network itself might be stable in its characteristics, the behaviour of the individual unit of interest, i.e. the farm remains unpredictable.

11.4 A glance beyond the scope

This study focusses on the slaughterhouse as sampling location for cattle for surveillance and monitoring programmes of bovine diseases. This gives a limited insight in the field of activity of veterinary public health or public health in general. The slaughterhouse is a very special location in the chain of animal production as it is the interface where live animals become food. Animal welfare is critical for transportation, pre-slaughter and during the slaughter process (Terlouw et al. 2008; Grandin 2010). Also, indicators for the welfare status on the farm of origin can be collected during the ante-mortem inspection and used for risk-based farm visits or lable programmes (Velarde & Dalmau 2012; Stärk et al. 2014).

Once the animal is dead, food-safety becomes the primary concern. Meat inspection is established since the 19th century (Edwards et al. 1997). Diseases and parasites that lead to visible leasions in organs or muscles are traditionally monitored during the post-mortem inspection. The detection of the first cases of bovine tuberculosis in Switzerland after over 50 years (Schiller et al. 2011) brought back to evidence the importance of well-trained meat inspectors for the safety of consumers. Other zoonotic agents like *Salmonella spp* or *Campylobacer* are monitored at the slaughterhouse in pigs and poultry (FSVO 2013).

Furthermore, analyses for the detection of residues of chemicals or antimicrobials in meat and meat products is conducted on a yearly basis according to EU law (Anonymous 1996), taking samples of different materials at the slaughterhouse. Recently, the demand for risk-based meat inspection increased (Calvo-Artavia et al. 2013; Sofos 2008; Presi et al. 2008; Alban et al. 2008) but is so far blocked by rigid legislative guidelines.

The early detection of emerging or re-emerging diseases is a essential task of the veterinary and public health authorities. Ongoing studies assess the use of data collected during meat inspection for syndromic surveillance (Vial & Reist 2014).

In a nutshell, the slaughterhouse can serve as data source for surveillance programmes and much more. The information available is of interest for animal and human health and the data collected by the veterinary service should be discussed with public health stakeholders on a instiutionalized basis (Dórea et al. 2014). With the introduction of an IT-system that allows the automated information exchange between slaughterhouses and central authorities, the first step is taken towards maximizing the gain of the available information. By further developping the system and collaborating with stakholders in the field, veterinary and human public service and policy makers, the potential of the slaughterhouse can be fully exploited.

11.5 Literature

Alban, L. et al., 2008. Towards a risk-based surveillance for Trichinella spp. in Danish pig production. *Preventive veterinary medicine*, 87(3-4), pp.340–57. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18649961 [Accessed September 22, 2014].

Anonymous, 2014. *Bericht zur Überwachung von Tierseuchen und Zoonosen*, Available at: http://www.blv.admin.ch/themen/02794/03039/index.html?lang=de&download=NHzLpZeg7t,lnp6I0NTU042l2Z6ln1acy4Zn4Z2qZpnO2Yuq2Z6gpJCGdoB7hGym162epYbg2c_JjKbNoKSn6A--.

Anonymous, 1996. *Egregio SCouncil directive 96/23/EG on measures to monitor certain substances and residues thereof in live animals and animal products*, Available at: http://ec.europa.eu/food/food/chemicalsafety/residues/council_directive_96_23ec.pdf.

Bigras-poulin, M., Thompson, R.A. & Chriel, M., 2006. Network analysis of Danish cattle industry trade patterns as an evaluation of risk potential for disease spread. *Preventive Veterinary Medicine*, 76, pp.11–39.

Bleul, U., 2011. Risk factors and rates of perinatal and postnatal mortality in cattle in Switzerland. *Livestock Science*, 135(2-3), pp.257–264. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1871141310004142 [Accessed August 21, 2014].

Blickenstorfer, S. et al., 2011. Using scenario tree modelling for targeted herd sampling to substantiate freedom from disease. *BMC veterinary research*, 7(1), p.49. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3170209&tool=pmcentrez&rendertype=abstract [Accessed May 27, 2013].

Calvo-Artavia, F.F., Nielsen, L.R. & Alban, L., 2013. Epidemiologic and economic evaluation of risk-based meat inspection for bovine cysticercosis in Danish cattle. *Preventive Veterinary Medicine*, 108, pp.253–261.

Cameron, A. & Baldock, F., 1998. A new probability formula for surveys to substantiate freedom from disease. *Preventive Veterinary Medicine*, 34(1), pp.1–17. Available at: http://apps.isiknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=3&SID=T1E4EnEpeDcn3FdfJ6k&page=1&doc=1 [Accessed April 25, 2013].

Cannon, R., 2001. Sense and sensitivity--designing surveys based on an imperfect test. *Preventive veterinary medicine*, 49(3-4), pp.141–63. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11311950.

Dórea, F. et al., 2014. Toward One Health: are public health stakeholders aware of the field of animal health? *Infection Ecology & Epidemiology*, 4. Available at: http://dx.doi.org/10.3402/iee.v4.24267.

Edwards, D., Johnston, A. & Mead, G., 1997. Meat Inspection: an Overview o f Present Practices and Future Trends. , pp.135–147.

Frössling, J. et al., 2012. Application of network analysis parameters in risk-based surveillance - examples based on cattle trade data and bovine infections in Sweden. *Preventive veterinary medicine*, 105(3), pp.202–208. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22265643 [Accessed September 22, 2014].

FSVO, 2013. *Nationaler Zoonosebericht Schweiz 2013 für die EFSA*, Available at: http://www.blv.admin.ch/themen/03605/04710/index.html?lang=de&download=NHzLpZeg7t,lnp6I0NTU042l2Z6ln1acy4Zn4Z2qZpnO2Yuq2Z6gpJCGdn18fGym162epYbg2c_JjKbNoKSn6A--.

Grandin, T., 2010. Auditing animal welfare at slaughter plants. *Meat Science*, 86, pp.56–65.

Kiss, I., Green, D. & Kao, R., 2006. Infectious disease control using contact tracing in random and scale-free networks. *Journal of the Royal Society Interface*, 3, pp.55–62.

Konschake, M. et al., 2013. On the robustness of in- and out-components in a temporal network. *PloS one*, 8(2), p.e55223. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3566222&tool=pmcentrez&rendertype=abstract [Accessed September 9, 2014].

Lynch, J. & Silva, P., 2013. Integrating animal health and food safety surveillance data from slaughterhouse control. *Revue scientifique et technique (International Office of Epizootics)*, 32(2), pp.409–416.

Martin, S., Shoukri, M. & Thorburn, M., 1992. Evaluating the health status of herds based on tests applied to individuals. *Preventive Veterinary Medicine*, 14(1-2), pp.33–43. Available at: http://linkinghub.elsevier.com/retrieve/pii/016758779290082Q.

Martinez-López, B., Perez, A. & Sánchez-Vizcaíno, J., 2009. Social Network Analysis. Review of Genera Concepts and Use in Preventive Veterinary Medicine. *Transboundary and emerging diseases*, 56, pp.109–120.

Mweu, M. et al., 2013. Temporal characterisation of the network of Danish cattle movements and its implication for disease control: 2000-2009. *Preventive veterinary medicine*, 110(3-4), pp.379–87. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23473852 [Accessed October 5, 2014].

Nöremark, M. et al., 2011. Network analysis of cattle and pig movements in Sweden : Measures relevant for disease control and risk based surveillance. *Preventive Veterinary Medicine*, 99, pp.78–90.

Perrin, J. et al., 2011. Analyse de la mortalité bovine en France de 2003 à 2009. *INRA Prod. Anim*, 24(3), pp.235–244.

Presi, P. et al., 2008. Efficiency of risk-based vs . random sampling for the monitoring of tetracycline residues in slaughtered calves in Switzerland. *Food Additives and Contaminants*, 25(5), pp.566–573.

Reber, A., Reist, M. & Schwermer, H., 2012. Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhino-tracheitis and bovine enzootic leucosis in Switzerland. *SAT*, 154(5), pp.189–197.

Reist, M., Jemmi, T. & Stärk, K., 2012. Policy-driven development of cost-effective, risk-based surveillance strategies. *Preventive veterinary medicine*, 105(3), pp.176–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22265642 [Accessed September 22, 2014].

Rivas, A. et al., 2003. Identification of geographic factors associated with early spread of foot-and-mouth disease. *American journal of veterinary research*, 64(12), pp.1519–1527.

Schelling, E. & Zinsstag, J., Transdisciplinary research and One Health. In J. Zinsstag et al., eds. *One Health The Theory and Practice of Integrated Helath Approaches*. Marston Book Services Ltd.

Schiller, I. et al., 2011. Bovine tuberculosis in Europe from the perspective of an officially tuberculosis free country: Trade, surveillance and diagnostics. *Veterinary Microbiology*, 151, pp.153–159.

Sofos, J., 2008. Challenges to meat safety in the 21st century. *Meat Science*, 78, pp.3–13.

Stärk, K. et al., 2014. Strengths and weaknesses of meat inspection as a contribution to animal health and welfare surveillance. *Food Control*, 39, pp.154–162. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0956713513005835 [Accessed October 2, 2014].

Terlouw, E. et al., 2008. Pre-slaughter conditions, animal stress and welfare: current status and possible future research. *animal*, 2.

Velarde, A. & Dalmau, A., 2012. Animal welfare assessment at slaughter in Europe: Moving from inputs to outputs. *Meat Science*, 92, pp.244–251.

Vial, F. & Reist, M., 2014. Evaluation of Swiss slaughterhouse data for integration in a syndromic surveillance system. *BMC veterinary research*, 10(1), p.33.

Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3922649&tool=pmcentrez&rendertype=abstract [Accessed October 6, 2014].

Woolhouse, M. et al., 2005. Epidemiological implications of the contact network structure for cattle farms and the 20-80 rule. *Biology letters*, 1, pp.350–352.

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13 Appendix 1: Geographical distribution of holdings in the Swiss cattle industry

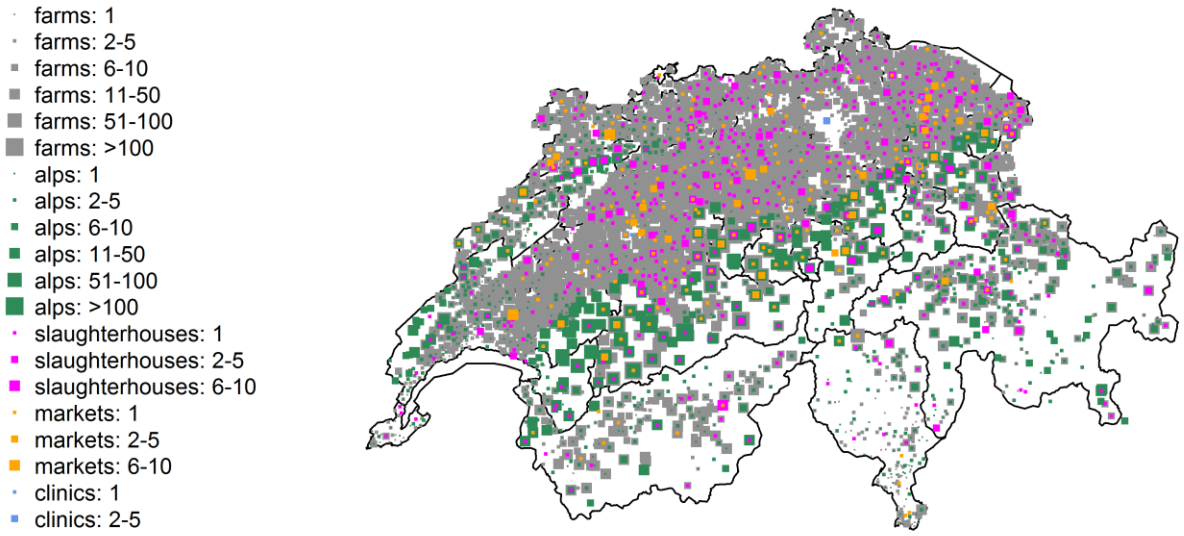

Figure A1.1: Holdings in the Swiss cattle industry per postal code area. Point size indicates number of holdings per category (see legend).

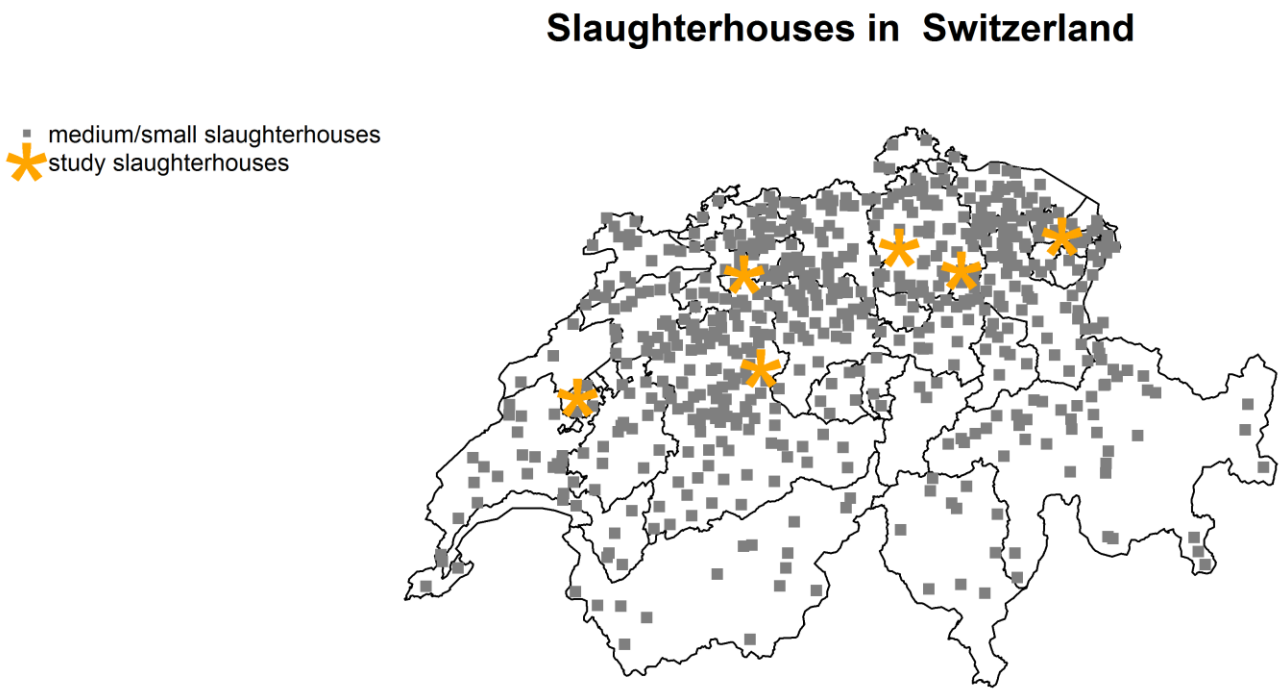

Figure A1.2: Cattle slaughterhouses in Switzerland. Orange asterisk: the 6 biggest cattle slaughterhouses. Grey dots: all other cattle slaughterhouses. The 6 biggest slaughterhouses are the study slaughterhouses for the feasibility study (Chapter 8) and the cost and sensitivity analysis (Chapter 10).

14 Appendix 2: Matrix model of the Swiss cattle population

Background

Recalling the dynamic population model of the Swiss cattle model (chapter 6, figure A2.1), the Swiss cattle population appears to be in equilibrium over the study period, with no visible growth trend.

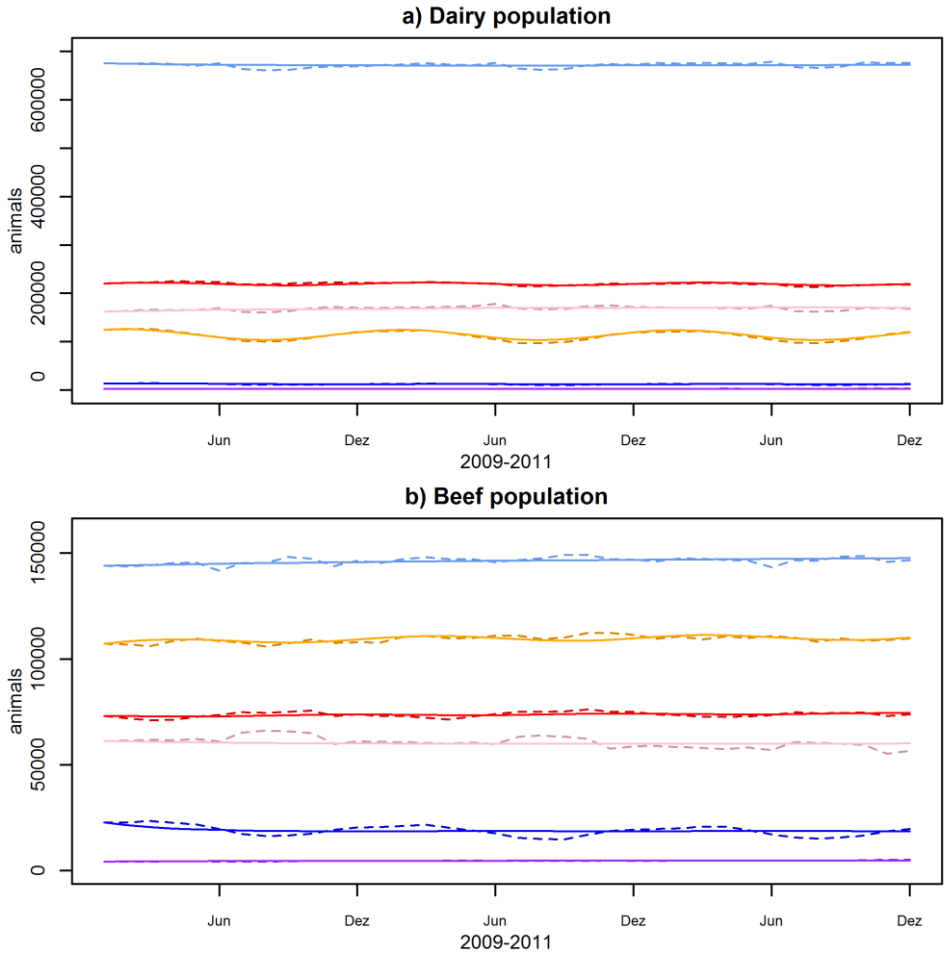

Figure A2.1: Animal numbers per age category. a) Dairy population. b) Beef population. Solid line: model data, dashed lines: AMD data. Light blue: cow, orange: male calf, red: female calf, pink: heifer, blue: young bull, purple: bull.

The fitted rates from the above mentioned model describe the population dynamic of the Swiss cattle population. We simulated the population using a projection matrix

model (Montshiwa 2007; Vandermeer & Goldberg 2003) to estimate the growth rate using a different methodological approach.

Methods

The theoretical concept of a demographic matrix model can be described as follows (see also Vandermeer & Goldberg 2003): the defined age and sex classes in the model define the population vector **N**. This vector is multiplied with the projection matrix **P** to obtain the population vector at the next time step. The projection matrix **P** consists of survival, fertility, mortality and slaughter and transition rates and describes the change in the population in every time step.

$$\mathbf{N}_{t+1} = \mathbf{N}_t * \mathbf{P} \quad (1)$$

Generalizing this equation it becomes:

$$\mathbf{N}_{t+n} = \mathbf{P}^n \mathbf{N} \quad (2)$$

After several iterations, the proportions of all age and sex classes stabilize at equilibrium. At the stable state the following equation applies

$$\mathbf{N}_{t+1} = \lambda * \mathbf{N}_t \quad (3)$$

where λ it is called dominant eigenvalue of **P** and the vector containing the proportion of each category is called eigenvector. Recalling the exponential equation

$$N_t = N_0 e^{rt} \quad (4)$$

we can deduct that $\lambda = e^r$ where r is the growth rate of the population.

λ can be approximated as the change in the total population per time step ($\mathrm{N}_t / N_{t\text{-}1}$) and becomes stable after several iterations; at this point, $\ln(\lambda) = r$ and the equilibrium population can be projected using equation 4.

For our model, the population vector **N** and the projection matrix **P** (see table 1 for the variable description) are as follows:

$$\mathbf{N} = \begin{pmatrix} X_{DF} \\ Y_{DF} \\ Z_{DF} \\ X_{DM} \\ Y_{DM} \\ Z_{DM} \\ X_{BF} \\ Y_{BF} \\ Z_{BF} \\ X_{BM} \\ Y_{BM} \\ Z_{BM} \end{pmatrix} \tag{5}$$

$$\mathbf{P} = \begin{pmatrix} sv_{XDF} & 0 & b_{XDF} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ t_{XDF} & sv_{YDF} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & t_{YDF} & sv_{ZDF} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & b_{XDM} & sv_{XDM} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & t_{XDM} & sv_{YDM} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & t_{YDM} & sv_{ZDM} & 0 & 0 & 0 & 0 & 0 & 0 \\ f_{XDF} & 0 & 0 & 0 & 0 & 0 & sv_{XBF} & 0 & b_{XBF} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & t_{XBF} & sv_{YBF} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & t_{YBF} & sv_{ZBF} & 0 & 0 & 0 \\ 0 & 0 & 0 & f_{XDM} & 0 & 0 & 0 & 0 & b_{XBM} & sv_{XBM} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & t_{XBM} & sv_{YBM} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & t_{YBM} & sv_{ZBM} \end{pmatrix} \tag{6}$$

The calculations were performed in MS excel and compared to values obtained with R (R Development Core Team 2008). A sensitivity analysis was conducted in R, using the package {popdemo}. The elasticity matrix or **P** was calculated to assess, which parameter has the most influence on λ and therefore the growth rate of the population.

We used 35 time steps (month) to simulate the same period as with the dynamic demographic model. The initial number of cattle in each category, the used rates and demographic segments are shown in table 1.

Table 1: fitted rates for the different age and sex classes from the dynamic population model (chapter 6); N: number of animals at t_0; b: birth rate; m: mortality rate; s: slaughter rate; t: transition rate to next age class; f: fattening rate, i.e. calves from the dairy system that are sold to beef farms for fattening; sv: survival rate, i.e. 1-(m+s+*t+f*).

category		N	b	m	s	t	f	sv
Dairy female calf	XDF	220‘418	0.0376	0.0094	0.0192	0.0680	0.0175	0.8859
Dariy heifer	YDF	162‘002		0.0007	0.0062	0.0802		0.9130
Dariy cow	ZDF	675‘285		0.0013	0.0190			0.9798
Dairy male calf	XDF	125‘129	0.0393	0.0256	0.1121	0.0206	0.0726	0.7691
Dairy young bull	YDF	13‘263		0.0017	0.1702	0.0235		0.8046
Dairy bull	ZDF	2‘505		0.0022	0.1113			0.8865
Beef female calf	XBF	73‘064	0.0331	0.0059	0.0390	0.0723		0.8828
Beef heifer	YBF	61‘254		0.0008	0.0262	0.0619		0.9111
Beef cow	ZBF	143‘987		0.0013	0.0234			0.9752
Beef male calf	XBM	107‘259	0.0353	0.0074	0.0637	0.0511		0.8777
Beef young bull	YBM	22‘747		0.0017	0.2828	0.0167		0.6987
Beef bull	ZBM	4‘178		0.0026	0.0637			0.9337

Results

With the projection in excel, λ was 1.000323 after 35 iterations (i.e. time steps) and r therefore 0.000323. The calculated value for λ in R was 1.000306 and r 0.000306.

The projection matrix model simulated the proportions of the animals per category nicely (figure 1)

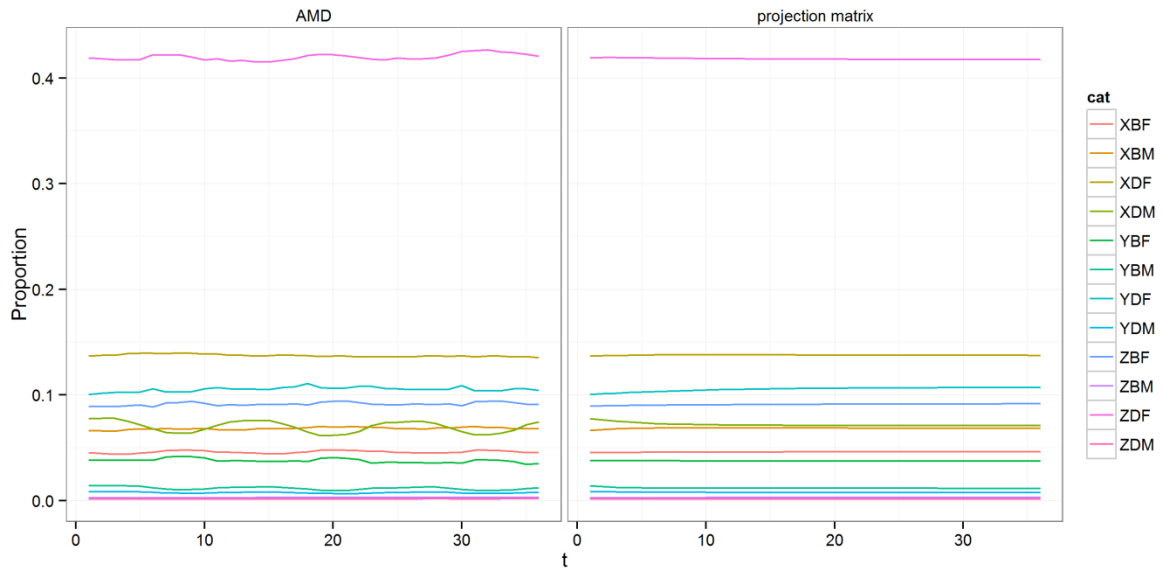

Figure 1: Proportion of animals in each category (for category names see table 1). After several time steps, the projected proportions stabilize. At this state, the proportions equals to the eigenvector (u) of the projection matrix P.

Simulating the number of animals in the total population with the described models, i.e. I) the dynamic population model (Vensim, chapter 6), II) the projection matrix, III) using the growth rate r deducted from II (r_PM) and IV) using the growth rate r calculated in R (r_R), the results show, that generally the growth rate is overestimated by the linear projections (figure 2).

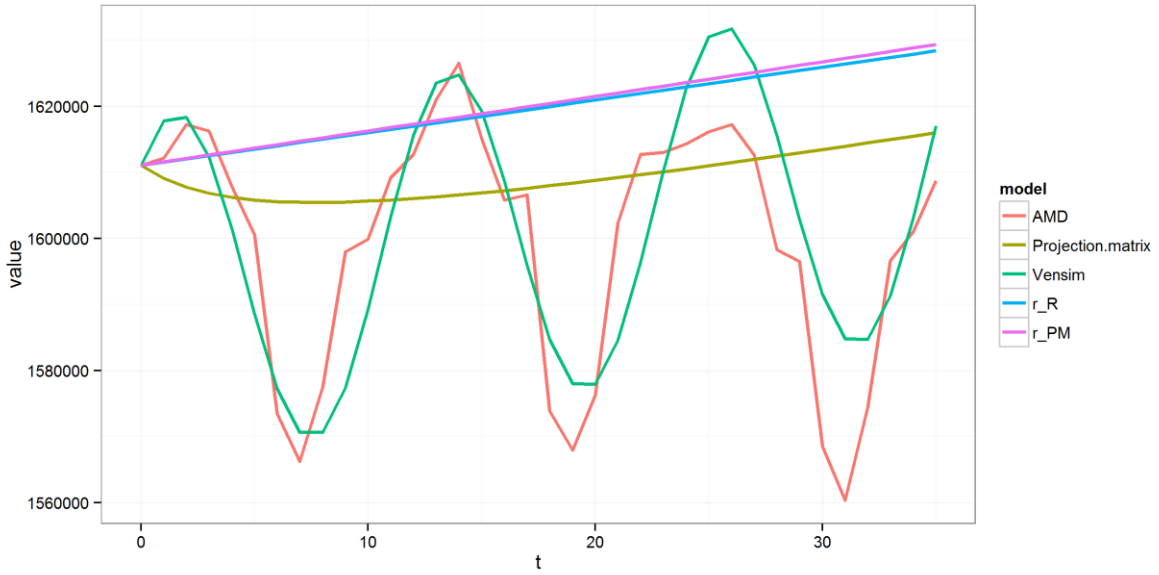

Figure 2. Comparison of three models of the total cattle population in Switzerland 2009-2011 (monthly time steps) and the observed data. AMD: animal movement database;

The sensitivity matrix of **P** the survival of adult dairy cows (sv_{ZDF}) contributed 70% of the overall sensitivity of λ. The only parameters contributing to the sensitivity of λ are sv_{XDF}, sv_{YDF}, t_{XDF}, t_{XDF} and $b_{XDF.}$.

Discussion

All used models assume that the demographic structure and development of the population in question is independent of the population density. The linear projection of the population (II-IV) has the mathematical foundation of exponential growth. Although the proportions of the categories in the simulations are comparable to the observed distribution, the growth rate is overestimated. The assumption of density independence is realistic in the sense that there is no resource limitation in terms of feed in a developed country like Switzerland. It is also probable, that the reproduction rate is maximized for economical reason. The offtake rate however, depends hugely on decisions and reasoning of the farmer. Mortality is influenced by his willingness to

invest in veterinary treatment; the fattening rate and the slaughter rate are influenced by the need of restocking in dairy herds and the milk and meat demand and prices on the market. In such a system, where all live events in an animal live are in the hand of its owners, models build and validated on natural processes might reach their limitation. Another advantage of the dynamic population model in comparison to the projection matrix model is the possibility to include non-linear processes. The birth rate for instance is not constant in the Swiss cattle population and has a distinct seasonality. This influences the whole population composition and might be one reason for the overestimated growth rate. Over a longer time period using yearly time steps, this effect might diminish. To include such seasonal effects, the modelled time span must be divided in short segments according the growth rate of the population i.e. >1 or <1 at the given time (Lesnoff et al. 2000).

The overwhelming importance of dairy cows for the demoghrapy of the Swiss cattle population in the sensitivity analysis using the matrix model concurred with the sensitivity analysis of the dynamic population model. However, the sensitivity analysis of the dynamic population model captured the relative importance of changes in other segments than dairy females with more detail (Chapter 6).

Literature

Lesnoff, M. et al., 2000. A steady-state approach of benefit ± cost analysis with a periodic Leslie-matrix model Presentation and application to the evaluation of a sheep-diseases preventive scheme in Kolda , Senegal. *Preventive veterinary medicine*, 46, pp.113–128.

Montshiwa, M.I., 2007. Leslie Matrix Model in Population Dynamics. *Sciences-New York*.

R Development Core Team, 2008. R: A language and environment for statistical computing. Available at: http://www.r-project.org.

Vandermeer, J. & Goldberg, D., 2003. *Population Ecology: First principles*, Princeton University Press.

15 Appendix 3: Defining the slaughter basin of the six biggest Swiss cattle slaughterhouse using EpiConactTrace

The slaughter basin of the big cattle slaughterhouses was specified, by calculating the monthly ingoing contact chain for the six biggest slaughterhouses using the R package EpiContactTrace (Nöremark & Widgren, 2014). The ingoing contact chain includes all farms, which reached one of the six slaughterhouses either by a direct animal movement or by indirect movements over one or several other holdings (figure A3.1). For every farm in the so defined network, the shortest path to any of the slaughterhouses was selected. Then the results were summarized by the 2946 postal code areas that hosted cattle in 2012 and expressed as monthly proportion of postal code areas covered.

The coverage of the six biggest cattle slaughterhouse by direct and indirect contacts for postal code areas is shown in table two. 103 (3.5%) postal code areas that hosted cattle at some point in 2012, are not in the slaughter basin of the big slaughterhouses in 2012 (figure A3.2).

The monthly coverage including only direct movements to the slaughterhouse varies from 68.9 to 77.95%. By including indirect contacts of two steps, the coverage is increased by roughly 10%. The geographical distribution over the year is shown in figure A3.3.

The inform gain for surveillance of ingoing cattle movement to farms in the sample, can somewhat be quantified by including also indirect movements to assess the slaughter basin.

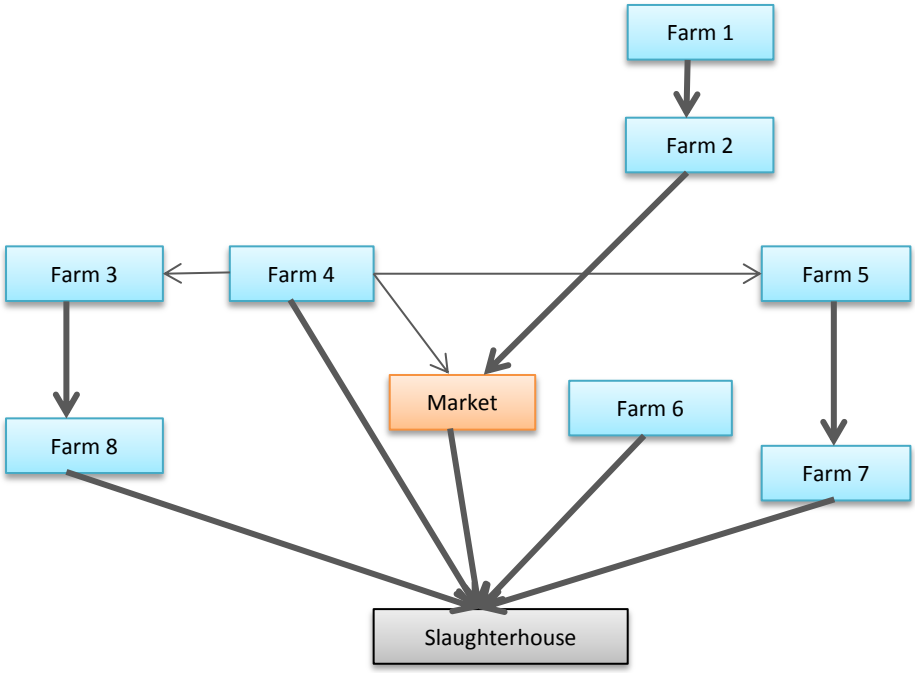

Figure A3.1: Schematic representation of the network to determine the slaughter basin. Thick lines are included for the calculation of the shortest path to the slaughterhouse. In the example, farm 4, 6, 7 and 8 have direct movements to the slaughterhouse, farms 2, 3 and 5 reach the slaughterhouse over two steps and farm 1 only if including also contacts over more than two steps.

Table 1: Slaughter basin of the six biggest cattle slaughterhouses in Switzerland 2012. The coverage is presented as percentage of postal code areas covered by direct contacts (1), direct contacts or contacts with one step in between (≤ 2) and all contacts included (all). The maximum numbers of steps to the slaughterhouse included for every month is given as maximum distance.

	Postal code areas covered [%]			
	distance to slaughterhouse			
	1	≤ 2	all	max distance
January	75.55	85.64	87.57	11
February	76.16	85.71	87.10	12
March	76.02	85.47	86.89	7
April	75.72	85.71	87.44	10
May	75.48	86.12	88.11	10
June	73.72	84.83	87.84	10
July	68.91	78.53	80.83	10
August	71.28	80.90	83.10	5
September	72.81	85.44	87.17	11
October	76.63	87.03	89.64	11
November	77.95	87.78	89.43	5
December	74.57	85.30	86.66	5
Average	74.57	84.87	86.82	

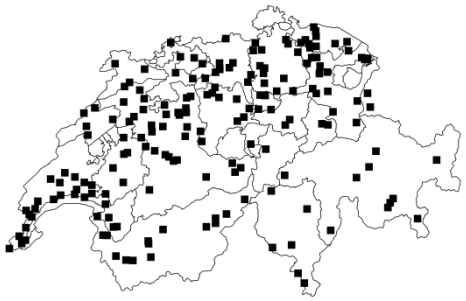

Figure A3.2: postal code areas with cattle but not in the slaughter basin of the six biggest Swiss cattle slaughterhouses in 2012.

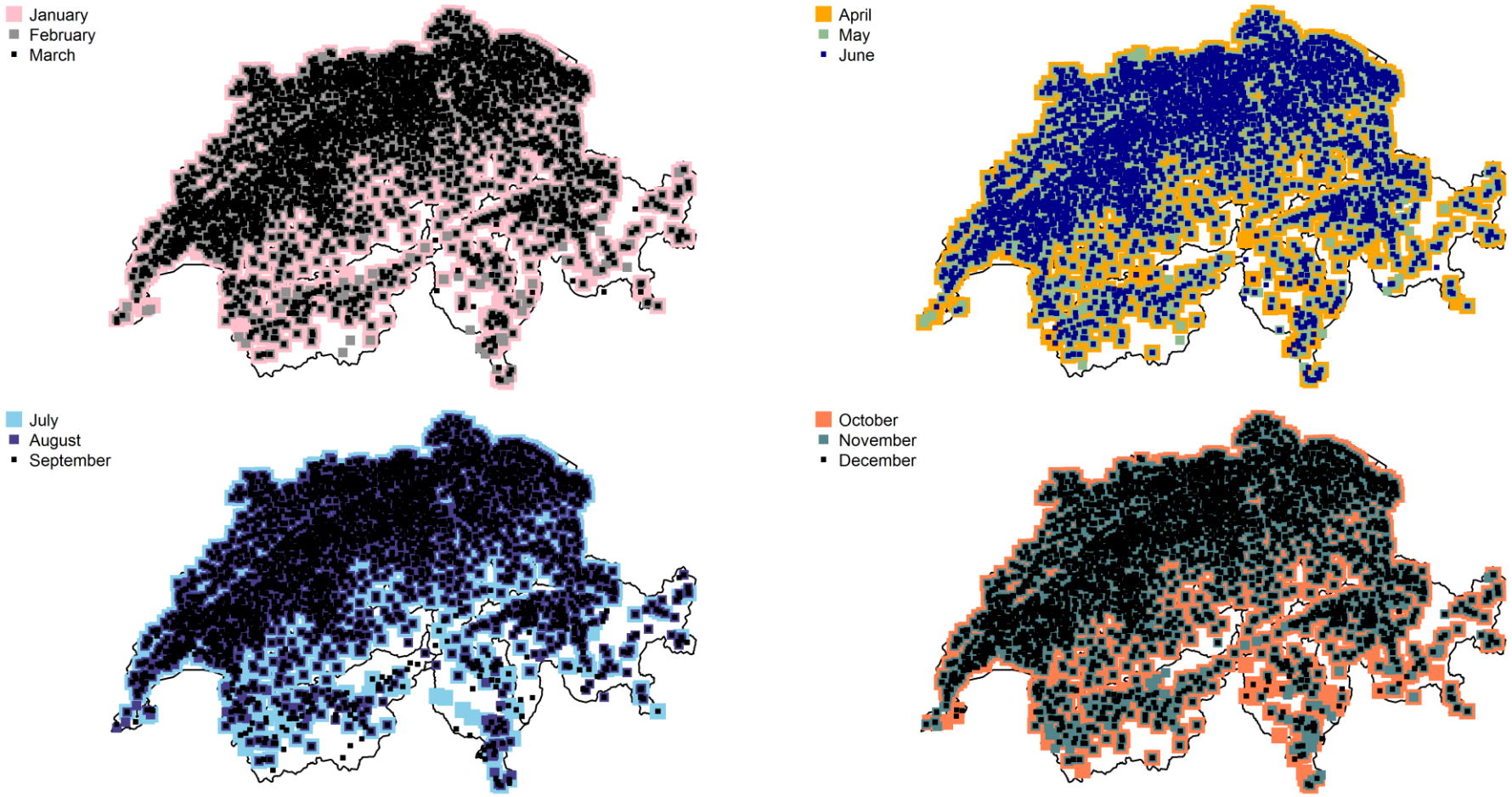

Figure A3.3: Geographical representation of the coverage by postal code areas for three-month periods. Dots indicate that the postal code area is represented in at least one slaughterhouse. The colours of the dots represent the month (see individual legends).

Literature

Nöremark, M., & Widgren, S. (2014). EpiContactTrace: an R-package for contact tracing during livestock disease outbreaks and for risk-based surveillance. *BMC Veterinary Research*, *10:71*. doi:10.1186/1746-6148-10-71

R Development Core Team, 2008. R: A language and environment for statistical computing. Available at: http://www.r-project.org.

16 Appendix 4: Surveillance of cattle populations - assessing practical and theoretical aspects of Swiss abattoirs as data sources

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Keywords: slaughterhouse surveillance, surveillance system

Abstract ICAHS 2014

Abstract

The aim of this study was to assess the feasibility and the added value of transferring the sampling of cattle for surveillance and monitoring programmes in Switzerland from on-farm to the slaughterhouse. The 5 biggest Swiss cattle slaughterhouses were included in a feasibility study and the animal movement database (AMD) served as data source for summary statistics and models regarding the slaughter population. The results underline the difficulty to draw conclusions at herd level in the slaughterhouse, especially when animals are not slaughtered in batches, as it is the case for Swiss cattle. Changes in the population composition due to movements and births allow only for a relatively short time window to gather a representative sample which contrasts with the long sampling period required to collect enough animals per farm in order to gain reasonable herd-level sensitivities.

Introduction

Early detection of disease, monitoring of present agents and verification of freedom from disease are described as key tasks of modern public veterinary services in order to allow international trade with animals and agricultural goods and to document the sanitary status of domestic livestock. However, large scale surveys on farms are very costly and time-consuming. New animal diseases and zoonosis emerge and require additional programmes even tough national budgets are reduced in many countries. To develop cost-effective tools for animal health surveillance is therefore a priority of decisions makers in the field of veterinary public health.

The major livestock species in Switzerland is cattle. To monitor the cattle population's health status, the veterinary authorities invest substantial resources in surveillance programmes including annual surveys. With the change from on-farm sampling of

individual blood samples to bulk tank milk testing as a cost-effective tool for dairy cattle surveillance in 2010 (Reber et al. 2012, Reist et al. 2012), the production type became an important criterion for shaping the sampling strategy of national surveillance programmes. Beef and fattening cattle, which represent one third of the population, still have to be sampled separately by conducting costly sampling on the farm. To gain efficiency in the non-dairy sector, the implementation of routine sampling of cattle at slaughterhouses is an alternative to on farm sampling.

Cattle are clustered in herds and a two stage sampling procedure is applied. Typically, surveys require conclusions on herd level (e.g. substantiation of freedom from infectious bovine rhinotracheitis (IBR) with 99% confidence at the 0.2% herd prevalence level).

In Switzerland, cattle are not slaughtered in batches and often insufficient numbers of animals from one herd are slaughtered on a single slaughtering day to achieve reasonable herd sensitivities to draw conclusions on herd level. To infer on herd level, animals of the same herd of origin have to be assignable to the farm, even if they arrive on different days at different slaughterhouses. This is even more relevant for risk-based surveys where animals from targeted farms at risk need to be preferentially sampled.

The presented study aimed at assessing the use of slaughterhouses in Switzerland for surveillance of cattle populations for different sampling strategies in two work packages:

- A feasibility study to evaluate the practicability of sampling in the slaughterhouse and to assess the possibility of selecting animals or farms according to given risk factors under current conditions

- A model to compare the prevalence in the population and a simulated slaughterhouse sample

Material and methods

First, a study was conducted to assess the feasibility of sampling animals exclusively from the beef production with the currently available tools and infrastructure. The feasibility study included the six largest cattle slaughterhouses. The study methods were on-site inspections, a workshop with stakeholders and a pilot study. Additionally, the bluetongue (BT) surveillance programme 2011, which was conducted in the slaughterhouses, was retrospectively evaluated relating to the sample selection of animals between 6 and 18 month of age and not more than 6 animals from one farm.

The spatial and temporal pattern of how Swiss farmers sent their cattle to abattoirs was studied by analyzing the data of the Swiss Animal Movement Database (AMD).

To compare prevalences in the population and a sample taken on slaughterhouse level, a model was built using the AMD data as basis. In this model, animals were randomly “tagged” in a two stage approach and then tracked if they occurred in the slaughter population. The proportion of tagged animals was interpreted as “disease prevalence” in the sampling population. The prevalences were compared for nine scenarios combining different between- and within-herd prevalences. The proportion of tagged animals in the slaughterhouse sample was followed over one year.

For data storage and extraction a PostgreSQL database was used. Summary statistics, the model and graphs were created in R.

Results

Feasibility: The discussions and guided visits with slaughterhouse veterinarians revealed several important points to consider when planning large scale sampling in the slaughterhouse; a wide variety of sampling material can be gained, but with the exception of blood, red organs and meat juice a technician has to be assigned exclusively for the sampling as most matrices are not accessible from the conventional workstations. The recognition of animals according to given criteria is very difficult, as at present only very limited information can be extracted directly from the accompanying document for individual animals. The only reliable criterion is calf/adult, as the size of carcass and organs are visually distinguishable. Sex, breed or production type are no longer recognizable once the skin is removed.

In the pilot study, selecting samples based on matching with the databases (AMD or slaughterhouse intern) after a rough pre-selection of presumably beef animals at the slaughter chain was inefficient. Personnel resources and sampling material were wasted since too many samples had to be discarded (209 of 1130, i.e. 18.5%) due to the poor pre-selection of suitable animals.

The evaluation of the BT survey based on information stored in the AMD revealed that from 3663 sampled animals 611 (16.7%) were older than 18 months and 15 (0.4%) younger than 6 months. Of the 1283 sampled farms, 71 (5.5%) had more than 6 animals in the sample. Forty-nine of these farms delivered cattle to more than one of the involved slaughterhouses and/or slaughtered at more than one day over the sampling period.

Generally, the Swiss farmers deliver their animals often in different abattoirs on scattered days for slaughter (Figure A4.1).

Prevalence comparison model: At the beginning of sampling the herd-level prevalence is underestimated in the slaughterhouse for all within- / between-herd prevalence combinations (Figure A4.2).

Over time, the proportion of positive herds in the slaughterhouse sample rises. This rise is partially due to a high rate of tagged animals being moved to previously negative herds (Figure A4.3) which explains the prevalence level above the initially set prevalence.

On animal level, the prevalences in the slaughterhouse sample are comparable to the introduced prevalence in the population for the first months then drops rapidly as no new animals are “tagged” (i.e. no disease transmission is simulated).

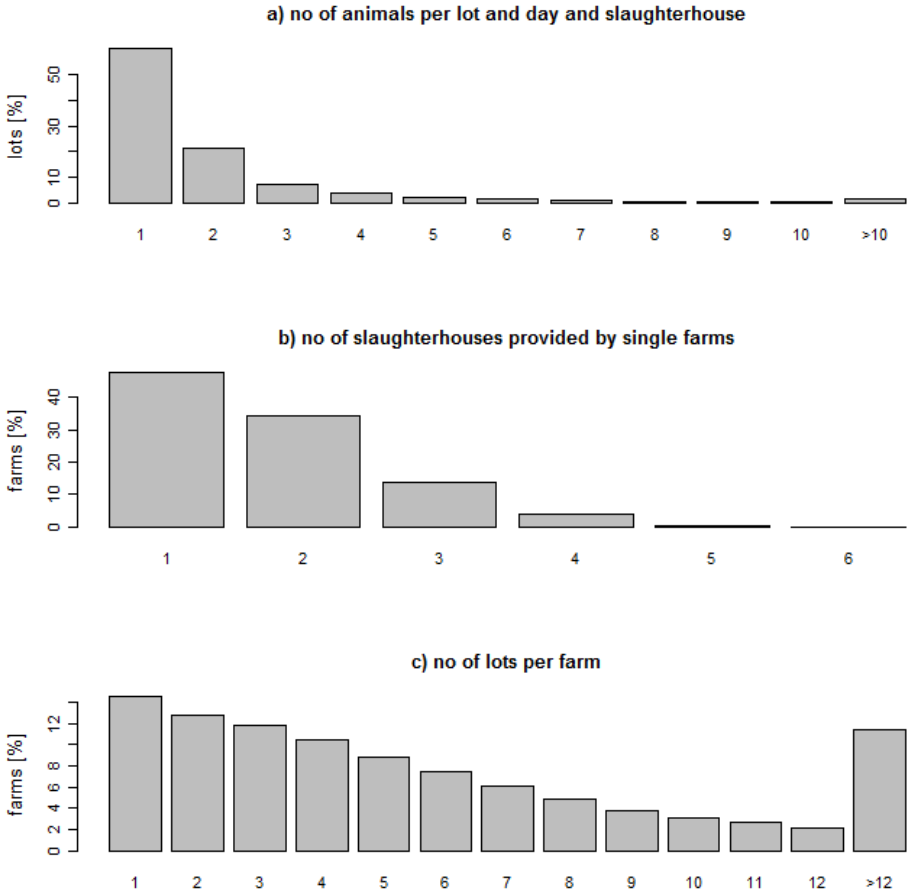

Figure A4.1: Cattle sent to slaughter from Swiss farms in 2009. A lot is defined as the number of animals that are delivered from one farm to one of the study slaughterhouses on one day. a) Number of animals per lot, slaughterhouse and day from one farm. b) Number of study slaughterhouses supplied by a single farm with at least one lot. c) Number of lots one farm brought to the study slaughterhouses over the year.

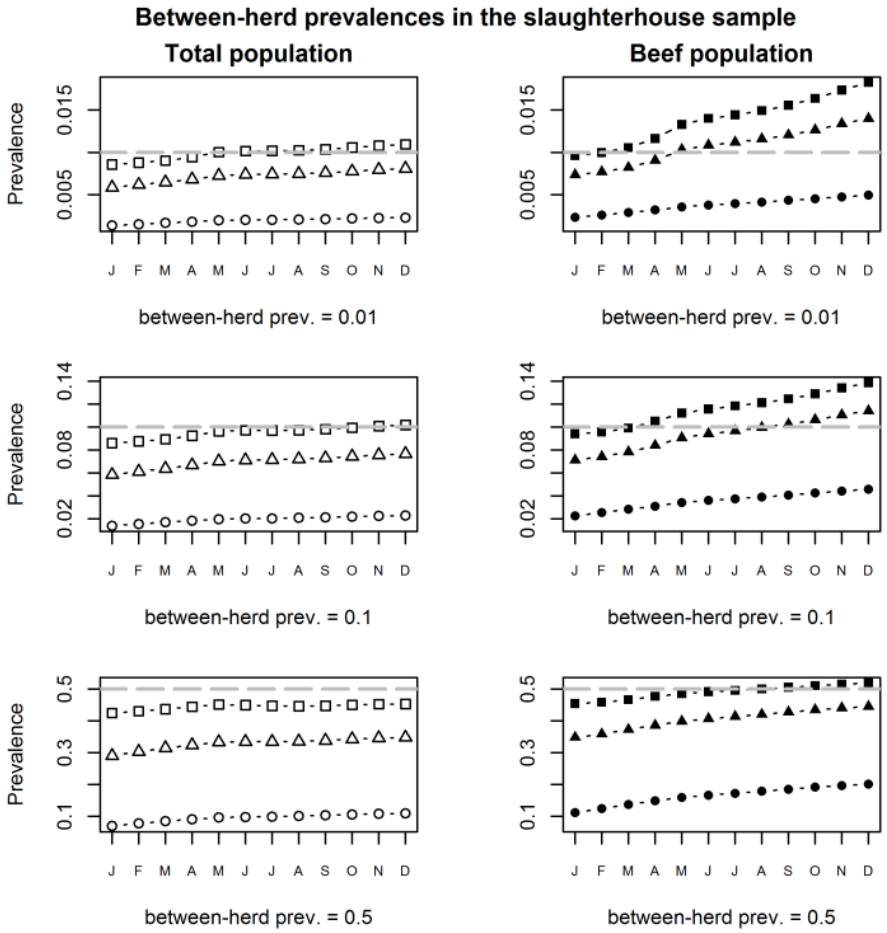

Figure A4.2: Herd level prevalence in the slaughterhouse sample. Prevalence in population at the time of tagging: grey dashed line; Within-herd prevalence 0.1: circles; Within-herd prevalence 0.5: triangles; Within-herd prevalence 0.8: squares; beef population: filled symbols.

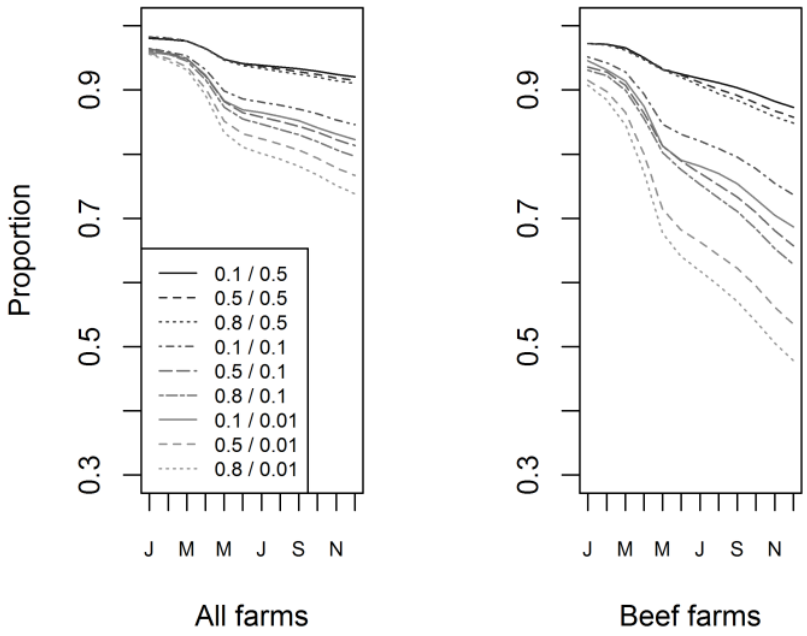

Figure A4.3: Mean proportion of originally tagged farms among the positive farms in the sample over time (January-December 2011) for all within- / between-herd prevalence combinations.

Discussion

The identification of animals according to sampling criteria is the limiting factor for better exploiting the slaughterhouses for the surveillance of the cattle population. In many mandatory monitoring programmes the herd is the unit of interest and several animals per herd have to be sampled. In Switzerland, cattle are gathered by traders on the farms and often only a few or single animals per farm are collected and delivered at the slaughterhouse. Additionally, the animals are not consistently announced to the slaughterhouses before their delivery and therefore the slaughterhouses cannot plan the sampling ahead.

Targeted sampling based on criteria on animal level is only achievable to a limited degree. Age categories, farm of origin, production type, breed and even the sex of the animals are difficult if not impossible to recognize at most of the positions relevant for sampling along the slaughter chain. Only the distinction of calves and older animals is visually feasible, although not precise (calves may vary in age, weight and size). If animals have to be selected according to given criteria, the suitable animals are most likely scattered over time and the sampling is only economically reasonable if it can be conducted at meat inspection. This is particularly important for constant surveillance applied during control programmes.

Results of the prevalence comparison model show that there is generally less underestimation of the herd-level prevalence in the beef population, which concurs with the presumption that beef animals are slaughtered more often in batches and therefore a better herd-level sensitivity is reached in the sample.

The rise in the herd-level prevalence over time points out the importance to consider animal movements for the planning and evaluation of surveillance programs with a longer duration. Even without newly infected animals, herds get the chance to test positive by the arrival of positive animals.

Over all, the model confirms the difficulties of drawing conclusions on herd level from sampling at the slaughterhouse (e.g. Ebel, 2008).

When the clustering of animals in farms is not a concern and the conclusions of a surveillance program can be made on animal level, the slaughterhouse gives a good estimate of the prevalence in the population, provided the probability of slaughter is not changed by the disease in consideration (e.g. subclinical infection).

With the given model settings, i.e. no disease transmission after the tagging or disease recovery or loss of antibodies, the prevalence in the sample equals the initial

prevalence for about 3 month and then drops rapidly. These results suggest that a cross sectional study conducted at the slaughterhouse should not take longer than a few months to represent the population at a given moment in time.

In conclusion, the relocation of the sampling of the cattle population to the slaughterhouses is possible for the entire population or only the beef population. However, without a centralized, AMD based data management system connected to the data management system of the slaughterhouse enabling real–time data exchange, and without technical aids such as visual signals to identify preselected animals, the risk-based or even herd-level sampling is practically impossible. According to the results from the prevalence comparison model, the sampling period should not exceed a few month, as otherwise the herd compositions have changed considerably due to births and trade. This stands in conflict with the scattered arrival of animals from one farm in the slaughterhouse, as the sampling of enough animals per farm to reach reasonable herd–level sensitivities takes time.

References

Ebel, E.D., Williams, M.S., Tomlinson, S.M., 2008. Estimating herd prevalence of bovine brucellosis in 46 U.S.A. states using slaughter surveillance. Prev Vet Med 85, 295-316.

Reber A., Reist M., Schwermer H., 2012. Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhinotracheitis and bovine enzootic leucosis in Switzerland. SAT, 154 Heft 5: 189-197.

Reist M., Jemmi T., Stärk K. D. C., 2012. Policy-driven development of cost-effective, risk-based surveillance strategies. Prev. Vet. Med. 105, 176-184.

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Conferences attended

DACh – Epidemiologietagung. September 2014, Zürich, Switzerland. Oral presentation: Rinderbewegungen in der Schweiz 2012 – Eine Quantitative Beschreibung und Schlussfolgerungen für die Tierseuchenüberwachung

International Conference on Animal Health Surveillance ICAHS. May 2014, Havanna, Cuba. Oral presentation: Surveillance of cattle populations - assessing practical and theoretical aspects of Swiss abattoirs as data sources

International Conference on Animal Health Surveillance ICAHS. May 2011, Lyon, France.

Journée d'épidemiologie AEEMA. May 2008, Paris, France. Oral presentation: Surveillance en fonction des risques de la fièvre catarrhale ovine (Bluetongue) en Suisse

Publications:

Schärrer, S., Widgren, S., Schwermer, HP., Lindberg, A., Vidondo, B., Zinsstag, J., Reist, M. (2015). Evaluation of farm-level parameters derived from animal movements for use in risk-based surveillance programmes of cattle in Switzerland. BMC Veterinary Research. 11:149, DOI: 10.1186/s12917-015-0468-8, http://www.biomedcentral.com/1746-6148/11/149

Schärrer, S., Schwermer, HP., Presi, P., Lindberg, A., Zinsstag, J., Reist, M. (2015). Cost and sensitivity of on-farm versus slaughterhouse surveys for prevalence estimation and substantiating freedom from disease. Prev Vet Med.;120(1):51-61. doi: 10.1016/j.prevetmed.2015.01.020. Epub 2015 Feb 11.

Schärrer, S., Presi, P., Hattendorf, J., Chitnis, N., Reist, M., Zinsstag, J. (2014). Demographic model of the Swiss cattle population for the years 2009-2011 stratified by gender, age and production type. PloS ONE , Published: October 13, 2014DOI: 10.1371/journal.pone.0109329

Schärrer, S., Fahrion, A., Lindberg, A., Reist, M., (2013). Feasibility of slaughterhouse sampling for surveillance of beef cattle in Switzerland. FLWI, 6:59-63.

Reist, M., Geser, N., Hächler, H., **Schärrer, S.,** Stephan, R. (2013): ESBL-Producing Enterobacteriaceae: Occurrence, Risk Factors for Fecal Carriage and Strain Traits in the Swiss Slaughter Cattle Population Younger than 2 Years Sampled at Abattoir Level. PLoS

ONE 8(8): e71725. doi:10.1371/journal.pone.0071725

Chaignat, V., Nitzsche, S., **Schärrer, S.,** Feyer, D., Schwermer, H., Thuer, B. (2010): Concentration of milk improves Bluetongue antibody detection by use of an indirect ELISA. Vet. Microbiol. Jul 14;143(2-4):179-83.

Schärrer, S., Chaignat, V., Schwermer, H., Hadorn, D., Schaffner, F., Breidenbach, E. (2008): Surveillance en fonction des risques de la fièvre catarrhale ovine (Bluetongue) en Suisse. Epidémiologie et Santé Animale, 53: 97-104.

Schwermer H., Chaignat V., Thür B., Hadorn D., **Schärrer S.,** Schaffner F., Breidenbach E. (2008): Das Überwachungsprogramm der Blauzungenkrankheit in der Schweiz. Schweiz. Arch. Tierheil., Bd.150 Heft 3: 129-132.